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A *Staphylococcus aureus* lipoteichoic acid (LTA) derived structural variant with two diacylglycerol residues

A. Stadelmaier,[a] I. Figueroa-Perez,[a] S. Deininger,[b] S. von Aulock,[b] T. Hartung[b] and R. R. Schmidt[a,*]

[a]*Fachbereich Chemie, Universität Konstanz, Fach M 725, D-78457 Konstanz, Germany*
[b]*Fachbereich Biologie, Universität Konstanz, Fach M 668, D-78457 Konstanz, Germany*

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Abstract—Based on 1,2-*O*-isopropylidene-*sn*-glycerol five chiral building blocks containing differently modified glycerol residues were required for the synthesis of the target molecule **2**. One of these building blocks is diacylglyceryl β-gentiobioside carrying a phosphite residue at 6b-*O* position. Ligation of these five building blocks led to the desired glycerol phosphate backbone to which D-alanyl residues were attached, thus generating after *O*-deprotection the target molecule **2**, a bisamphiphilic structural variant of *Staphylococcus aureus* LTA. This compound displayed higher potency in terms of cytokine release by human blood leukocytes than the monoamphiphilic variant LTA.

1. Introduction

For Gram-negative bacteria, LPS is well established as the crucial stimulus of the innate immune system, as injection of LPS into mice causes all known symptoms of sepsis.[1] There is now good evidence that lipoteichoic acid (LTA) from the cytoplasmic membrane of Gram-positive bacteria is an immunostimulatory Gram-positive counterpart to LPS.[2–6] The most frequently isolated Gram-positive pathogen that causes infections is *Staphylococcus aureus*,[7] and the development of antibiotic resistance in this species is a big problem.[8,9] Therefore, alternatives and adjuvants to antibiotics are required. To identify these, it is important to understand the pathophysiology of this bacterial infection.

The structure of the *S. aureus* LTA is shown in Scheme 1.[3,10,11] For unequivocal bioactivity assignment, besides an improved isolation procedure,[11] the chemical synthesis of the structurally closely related compound **1a** (Scheme 1) was decisive.[3–5,12] This compound contains the hydrolytically labile D-alanine residues in the required ratio with other substituents at a hexameric glycerophosphate backbone. Compound **1a** exhibited essentially the same biological activity in terms of initiation of cytokine release by human blood leukocytes as found for the natural product. Whereas the replacement of the D-alanine residues by L-alanine residues as in **1b** led to almost complete loss of biological activity.[4] Further structural modifications of LTA **1a**, as for instance deletion of the gentiobiose moiety or replacement of the hydrolytically labile ester bond to the D-alanyl residue by a stable amide bond, led only to minor decrease of induction of cytokine release by human blood leukocytes.[5,13] Thus, the importance of the D-alanyl residues was displayed. However, none of the investigated structural modifications so far led to an increased induction of cytokine release. For this, optimal presentation of the hydrophilic part of LTA to the receptor should be of utmost importance; therefore, bisamphiphilic compound **2** (Scheme 2) having two diacylglycerol gentiobioside residues at each end of the glycerophosphate backbone was designed. It was hypothesized that with two lipid anchors, possibly within the same membrane, the epitope presentation should be supported due to sterically improved accessibility to the D-alanyl and the α-O-linked *N*-acetylglucosamine residues.[14]

2. Results and discussion

The retrosynthesis of compound **2** is shown in Scheme 2. Disintegrations ①–④ lead to building blocks **3** to **8** which

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* Corresponding author. Tel.: +49 7531 88 2538; fax: +49 7531 88 3135; e-mail: Richard.Schmidt@uni-konstanz.de

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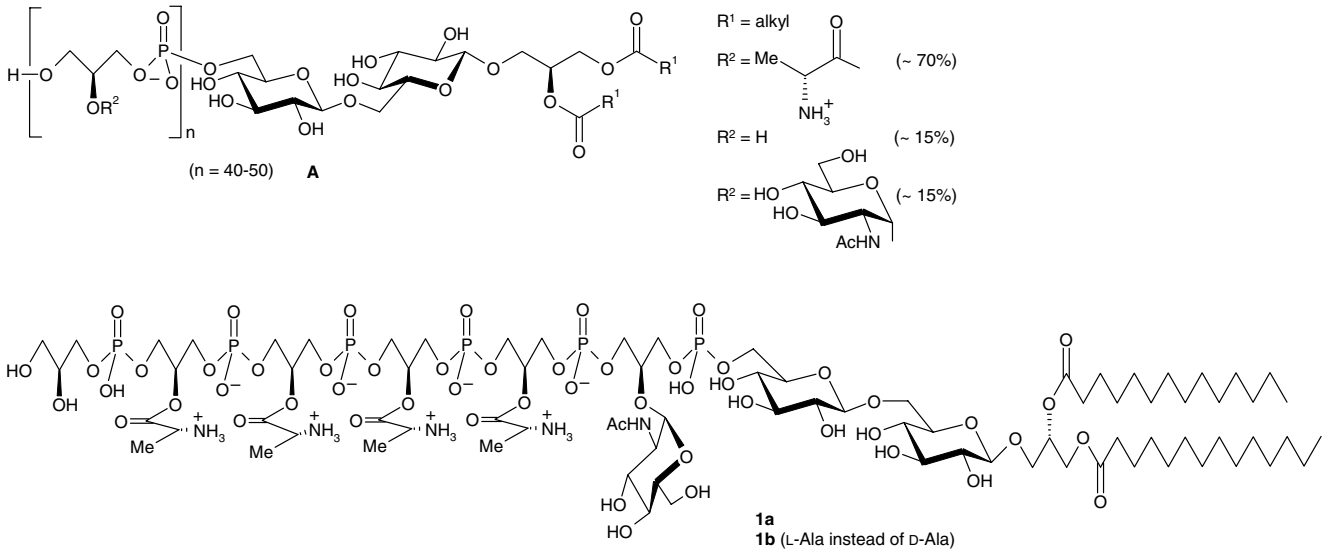
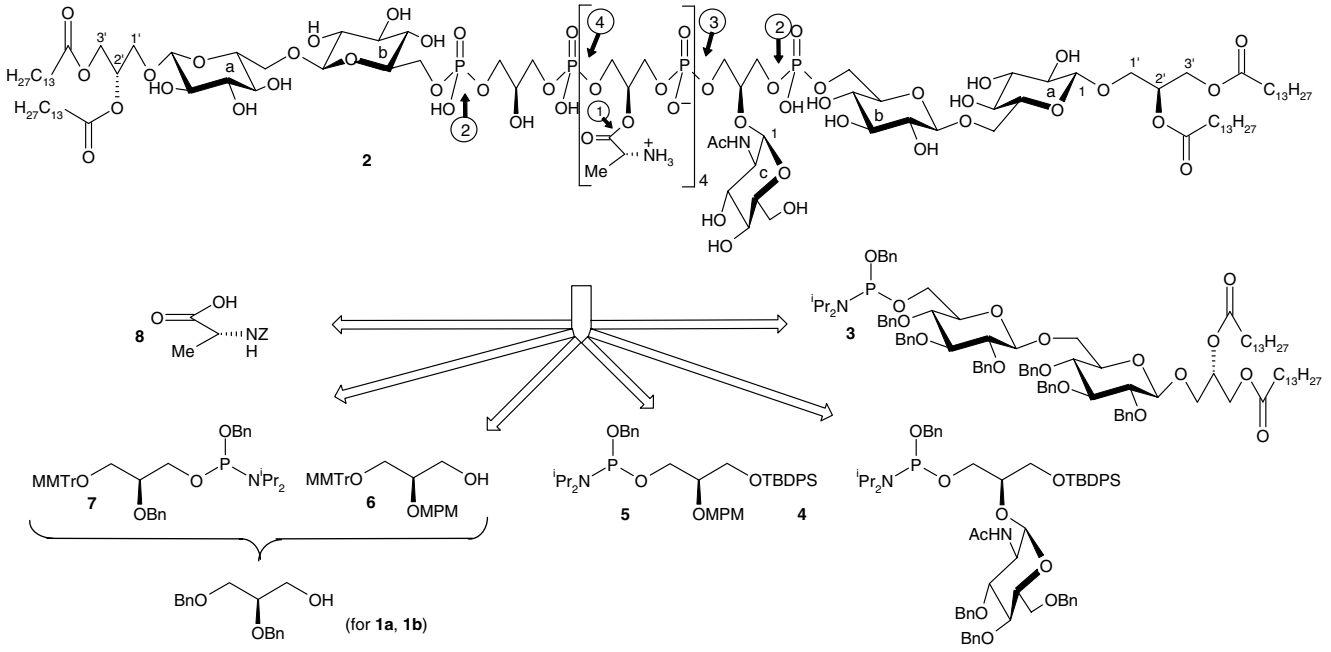


Scheme 1. General structure **A** of lipoteichoic acid (LTA) from *Staphylococcus aureus* (**A**) and structure of closely related compound **1a** and its diastereoisomer **1b**.

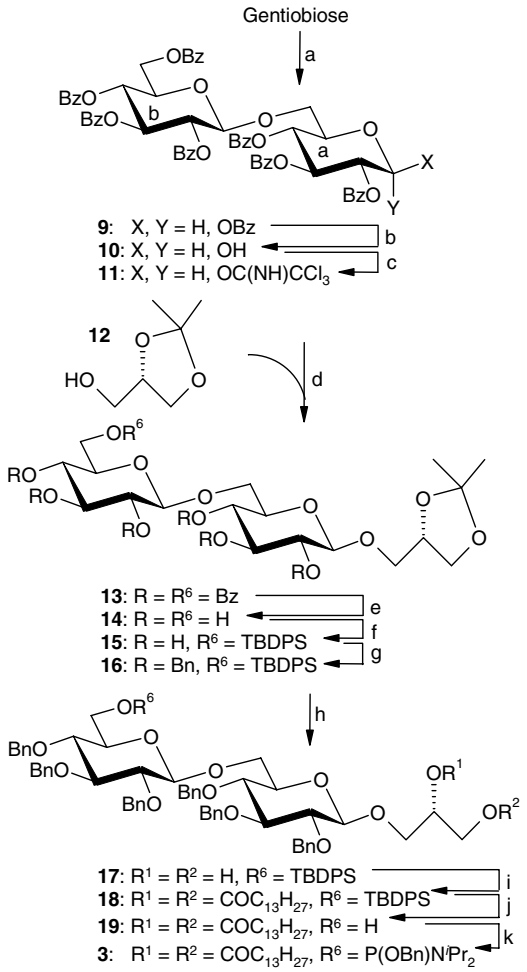


Scheme 2. Structure of target molecule **2** and required building blocks **3**–**8** for the synthesis.

consider the presence of diacylglyceryl gentiobioside, glyceryl 2-*N*-acetylamino-2-deoxy-α-D-glucopyranoside, D-alanylated glycerol and 2-O-unsubstituted glycerol residues, respectively, and their sequence specific linkage via phosphorus diester bonds. Also the most important aspect, the lability of the D-alanyl residues, which are readily cleaved at pH 8.5, is taken into account: as temporary protecting groups in building blocks **5** and **6** 4-methoxyphenylmethyl (MPM) groups are chosen; they can be selectively cleaved by oxidation after completion of the backbone synthesis. Ensuing attachment of D-alanyl residues with Z-protected alanine **8** and then complete O-debenzylation will provide the target molecule **2**. Building blocks **3**–**5** and, as substitute for **6** and **7**, 2,3-di-*O*-benzyl-*sn*-glycerol,[4] were successfully employed in the synthesis of LTAs **1a**, **b**. Because this work has not been reported in detail,[15] the syntheses of compounds **1a**, **b** will also be described.

2.1. Synthesis of building block 3

Benzoylation of gentiobiose with benzoyl chloride in pyridine gave an α-, β-mixture of per-O-benzoylated compound **9** (Scheme 3). Treatment of **9** with hydrazinium acetate in DMF permitted chemoselective removal of the anomeric *O*-benzoyl group furnishing 1-O-unprotected compound **10**. Reaction of **10** with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene



Scheme 3. Synthesis of building block **3**. Reagents and conditions: (a) Bz-Cl, pyr, 40 °C (98%); (b) N_2H_4·HOAc, 50 °C, DMF (78%); (c) CCl_3CN, DBU, CH_2Cl_2 (97%); (d) BF_3·OEt_2, CH_2Cl_2, −30 °C (80%); (e) NaOMe, MeOH (qu); (f) TBDPS-Cl, pyr, −15 °C (91%); (g) Bn-Br, NaH, DMF (66%); (h) HOAc, THF/H_2O, 80 °C (78%); (i) myristoyl chloride, NEt_3, THF, 50 °C (81%); (j) TBAF, HOAc, THF, 40 °C (76%); (k) $BnOP(N^iPr_2)_2$, tetrazole, THF/CH_2Cl_2 (79%).

(DBU) as base afforded trichloroacetimidate **11** as a 1:1 anomeric mixture. Glycosylation of 1,2-*O*-isopropylidene-*sn*-glycerol (**12**)[16] with **11** in the presence of BF_3·OEt_2 as activator gave exclusively β-glycoside **13** in 80% yield. Complete O-debenzoylation with sodium methoxide in methanol afforded compound **14** which was regioselectively silylated at the primary hydroxy group with *tert*-butyldiphenylsilyl (TBDPS) chloride in pyridine to give 6b-O-protected compound **15**. Following O-benzylation with benzyl bromide and sodium hydride as base in DMF as solvent afforded fully O-protected gentiobioside **16**. Acid-catalyzed O-deisopropylidenation (→ **17**) and then treatment with myristoyl chloride in the presence of triethylamine as base led to introduction of two myristoyl residues furnishing compound **18**. 6b-O-Desilylation with tetrabutylammonium fluoride (TBAF) in the presence of acetic acid in THF as solvent gave 6b-O-unprotected compound **19** which on reaction with benzyloxy-bis(diisopropylamino)-phosphane[17] in the presence of tetrazole as catalyst furnished building block **3**.

2.2. Synthesis of LTA 1a and its diastereomer 1b

With building block **3** and the previously synthesized glycerophosphate oligomer **20**,[13] possessing the required protecting group array for regioselective chain extension and for following D-alanyl residue attachment, the synthesis of **1a**, **b** could be readily completed (Scheme 4). Ligation of **3** and **20** in the presence of tetrazole and then oxidation with *tert*-butylhydroperoxide gave phosphate linked intermediate **21**, which contains the backbone of the target molecule. Treatment of **21** with ceric(IV) ammonium nitrate (CAN) liberated four of the glycerol hydroxy groups affording compound **22**. Attachment of the Z-protected D-alanyl residues was performed with excess **8** in the presence of (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate[18] (PyBOP)/*N*-methyl-imidazole as condensing agent to give the fully protected target molecule **23a**. Hydrogenolysis with Pearlman's catalyst[19] in a mixture of CH_2Cl_2/MeOH/H_2O (5:5:1) furnished the desired final product **1a** after hydrophobic interaction (HI) chromatography on octylsepharose in 47% yield. The structural assignment was based on MS and NMR data and comparison with naturally occurring material.[3] Compound **22** was similarly transformed with Z-protected L-alanine via **23b** into **1b**.

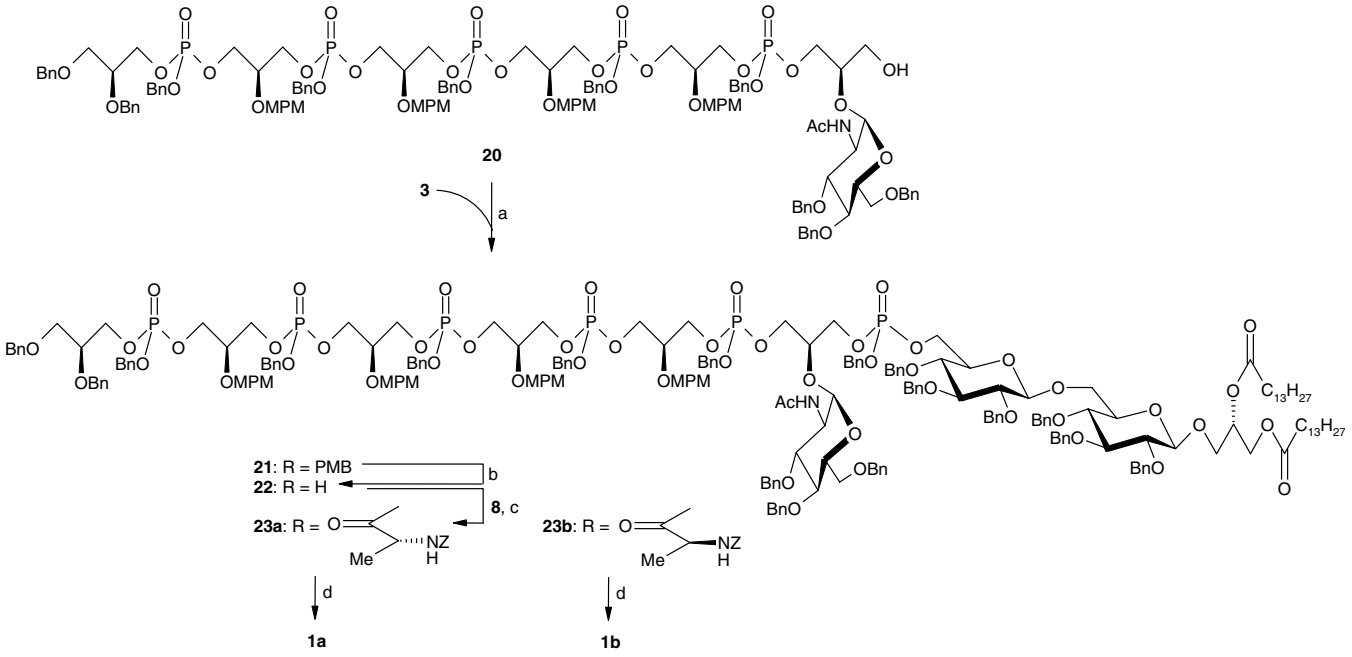
2.3. Synthesis of building blocks 6 and 7

Previously described 1-*O*-(*tert*-butyldiphenylsilyl)-2-*O*-(4-methoxybenzyl)-*sn*-glycerol (**24**),[13] obtained from 1,2-*O*-isopropylidene-*sn*-glycerol, was treated with monomethoxytrityl (MMTf) chloride in CH_2Cl_2/pyridine to afford fully O-protected glycerol derivative **25** (Scheme 5). Regioselective *O*-desilylation with TBAF in THF afforded the 1-O-unprotected building block **6**.

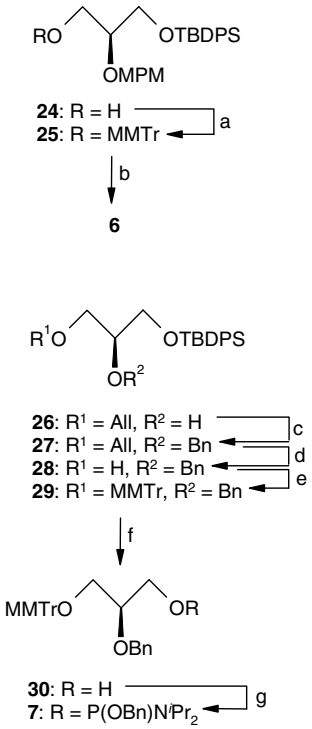
Treatment of previously described 3-*O*-allyl-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**26**)[13] with benzyl bromide in DMF afforded per-O-protected glycerol **27**. O-Deallylation with Wilkinson's catalyst[20] in the presence of DBU as base in ethanol furnished the 3-*O*-propenyl derivative which was cleaved with 1 M HCl in acetone to afford 3-O-unprotected **28**. Reaction with MMTr-Cl in CH_2Cl_2/pyridine furnished fully protected glycerol **29** which on treatment with TBAF in THF led to 1-O-unprotected glycerol **30**. Phosphitylation with benzyloxy-bis(diisopropylamino)phosphane[17] led to the desired phosphite derivative **7**.

2.4. Synthesis of target molecule 2

The assembly of target molecule **2** from building blocks **3**–**8** was performed stepwise in solution starting with building block **6** (Scheme 6). Phosphitylation of **6** with **5** in the presence of tetrazole and then oxidation with *tert*-butylhydroperoxide gave phosphate intermediate **31** which was O-desilylated with TBAF in THF as solvent to furnish **32** with a free hydroxy group as acceptor for the next reaction cycles for chain extension. This way, with **5** intermediates **33**–**35** were obtained. Following cleavage of the MMTr group with camphersulfonic acid (CSA) in methanol afforded **36** which on phosphitylation



Scheme 4. Synthesis of compounds **1a** and **1b**. Reagents and conditions: (a) tetrazole, CH_2Cl_2; *t*-BuO_2H (75%); (b) CAN, MeCN/Tol/H_2O, −10 °C → rt (67%); (c) PyBOP, Me-Im, CH_2Cl_2 (**23a**, 70%; **23b**, 62%); (d) $Pd(OH)_2$/C, H_2, CH_2Cl_2/MeOH/H_2O (**1a**, 47%; **1b**, 40%).

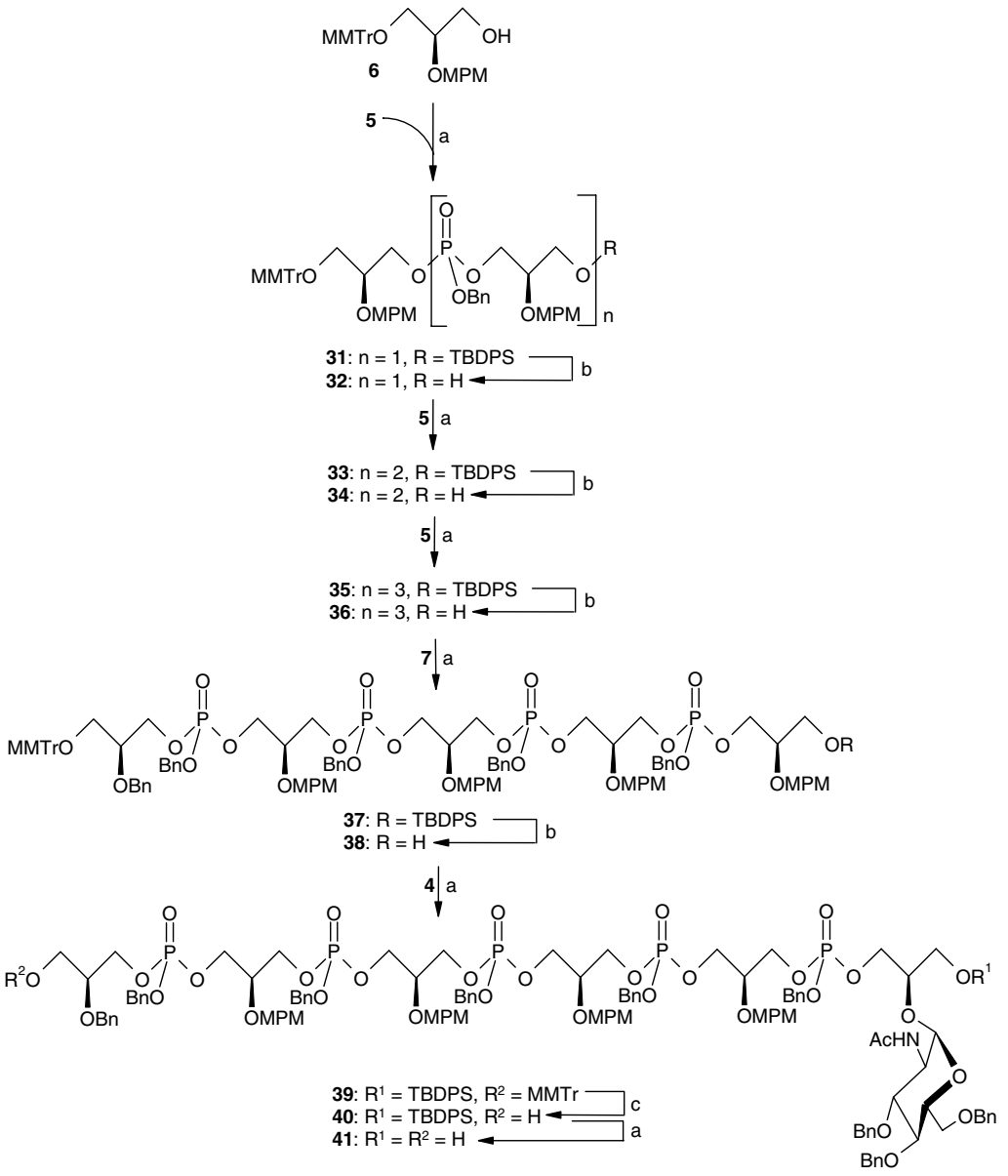


Scheme 5. Synthesis of building blocks **6** and **7**. Reagents and conditions: (a) MMTr-Cl, CH_2Cl_2/pyr (98%); (b) TBAF, THF (96%); (c) Bn-Br, NaH, DMF (72%); (d) $(Ph_3P)_3RhCl$, DBU, EtOH; HCl, Me_2CO, 70 °C (76%); (e) MMTr-Cl, CH_2Cl_2/pyr (98%); (f) TBAF, THF (98%); (g) $BnO(^iPr_2N)_2P$, tetrazole, THF/CH_2Cl_2 (qu).

with building block **7**, oxidation and then TBDPS group cleavage led to compounds **37** and **38**. Then phosphitylation with building block **4** and oxidation gave pentaphosphate intermediate **39**. Selective deprotection of the primary hydroxy groups first by treatment with CSA in methanol (→ **40**) and then with TBAF in THF afforded compound **41** which was available to attachment of building block **3** at each terminus. To this end, **41** was treated with tetrazole under standard conditions to afford compound **42** in good yield (Scheme 7), which contains the desired glycerophosphate backbone of target molecule **2**. For the attachment of the D-alanyl residues, the four MPM groups were selectively removed by treatment with CAN in an acetonitrile/toluene/water mixture as solvent affording compound **43** in 76% yield. D-Alanylation was performed with triethylammonium salt of **8** in the presence of *N*-methyl-imidazole and PyBOP as condensing agent furnishing fully protected target molecule **44** in 49% yield. Hydrogenolytic O-debenzylation (i.e. cleavage of 27 *O*-benzyl groups) was performed with Pearlman's catalyst[19] in a dichloromethane/methanol/water mixture as solvent. The crude product was purified by HI chromatography to afford target molecule **2** in 32% yield. Compound **2** was structurally ascertained as all intermediates by NMR and MS data and most intermediates also by elemental analyses.

3. Biological activity

The evaluation of the biological activity of compound **2** was performed in comparison with compound **1a** (Fig. 1), which induces a similar pattern of cytokine release as natural *S. aureus* LTA.[3,4] Measurement of initiation of cytokine release (A, TNFα; B, IL-8) by human blood leukocytes displayed that bisamphiphilic **2** is more potent than monoamphiphilic LTA **1a** by a factor of about 10. Hence, the hypothesis that bisamphiphilic LTA analogues should exhibit stronger biological activity than monohomophilic LTA **1a** was proven to be correct.



Scheme 6. Synthesis of glycerophosphate oligomer **41**. Reagents and conditions: (a) tetrazole, CH_2Cl_2; *t*-BuO_2H (**31**, 98%; **33**, 96%; **35**, 94%; **37**, 96%; **39**, 96%); (b) TBAF, THF (**32**, 82%; **34**, 91%; **36**, 92%; **38**, 95%; **41**, 85%); (c) CSA, MeOH (92%).

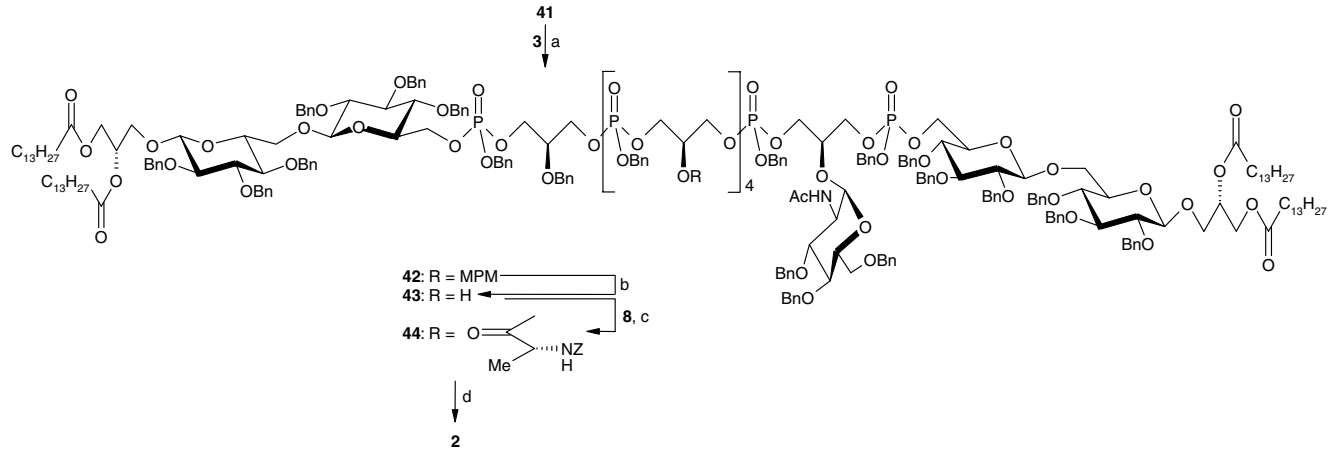
4. Conclusion

In conclusion, retrosynthesis of bisamphiphilic structural variant **2** of *S. aureus* LTA led to six building blocks out of which five required differently modified chiral glycerol residues. They readily permitted the assembly of compound **2**. The backbone construction was based on the ligation of different glycerol phosphites possessing, similar to nucleotide synthesis, temporary O-protecting groups permitting sequence specific chain extension. Hence, based on this synthesis design ready access not only to monoamphiphilic *S. aureus* LTA **1a**, which is also described in detail, but also to a multitude of structural variants of LTA is available. As anticipated, bisamphiphilic compound **2** is more potent in terms of induction of cytokine release in human leukocytes than natural LTA and its shortened version **1a**.

5. Experimental

5.1. General remarks

Solvents were dried according to standard procedures. NMR spectroscopic measurements were performed at 22 °C with Bruker DRX600 and Bruker AC250 instruments. TMS or the resonances of the deuterated solvents were used as internal standard. $CDCl_3$ ($\delta = 7.24$ ppm) was used as external standard; 85% of phosphoric acid was used as external standard for ^{31}P spectra. MALDI mass spectra were recorded with a Kratos Kompact Maldi II spectrometer; 2,5-dihydroxybenzoic acid (DHB) or *p*-nitroaniline and NaI were used as matrices for positive measurements, and trihydroxyacetophenone (THAP) was used as matrix for negative mode measurements. Optical rotations were measured with a Perkin Elmer polarimeter 241/MS in a 1-dm cell at 22 °C.



Scheme 7. Synthesis of target molecule **2**. Reagents and conditions: (a) tetrazole, CH_2Cl_2; *t*-BuO_2H (71%); (b) CAN, MeCN/Tol/H_2O, −10 °C → rt (76%); (c) PyBOP, Me-Im, CH_2Cl_2 (49%); (d) $Pd(OH)_2$/C, H_2, CH_2Cl_2/MeOH/H_2O (16%).

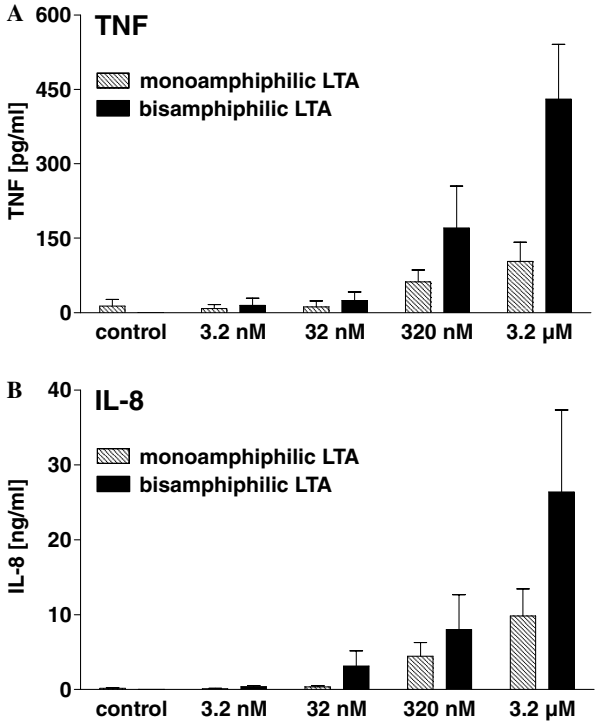


Figure 1. Concentration dependence of TNFα (A) and IL-8 (B) release in human whole blood in response to **1a** and **2** as measured by ELISA. Data are means (±SEM) of blood from four donors. SEM, standard error of the mean.

Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} plastic plates. Compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (400 mL). Flash chromatography was performed on J. T. Baker silica gel 60 (0.040–0.063 mm) at a pressure of 0.3 bar. Target molecules were purified by Hydrophobic Interaction Chromatography on octylsepharose as stationary phase and as elution phase was used as a gradient of propanol (15–60%) in 0.1 M ammonium acetate buffer (pH 4.8).

5.2. General procedure for phosphate formation

The alcohol and the phosphane (1.2 equiv) were coevaporated with dry CH_2Cl_2 and dried in high vacuum for 1 h. The mixture was dissolved in dry CH_2Cl_2 and tetrazole (2.5 equiv, previously dried for 1 h in high vacuum) was added. The reaction mixture was stirred for 1.5 h at room temp (TLC control), and after this time *t*-BuO_2H (1.3 equiv of 5.5 M solution in decane) was added. The reaction mixture was stirred for another 30–45 min and diluted with EtOAc, washed with saturated $NaHCO_3$ solution. The organic phase was dried over $MgSO_4$ and evaporated in vacuo. Purification by flash chromatography on silica gel gave the desired product.

5.3. General procedure for the removal of *tert*-butyldiphenylsilyl protecting group

The silylated compound was dissolved in THF (p.a. quality) and treated with TBAF (1.2 equiv of 1 M solution in THF). The reaction mixture was stirred for 30–45 min at room temp (monitoring by TLC). After this time, the reaction mixture was diluted with EtOAc and washed with saturated NH_4Cl solution and water. The organic phase was dried over $MgSO_4$ and the solvent evaporated in vacuo. Flash chromatography on silica gel gave the desired compound.

5.4. 6-*O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-1,2,3,4-tetra-*O*-benzoyl-α/β-D-glucopyranose (9)

Gentiobiose (14.13 g, 41.28 mmol) was dissolved in pyridine (375 mL), benzoyl chloride was added (50 mL, 0.48 mol) and the reaction mixture was stirred at 40 °C overnight. The solvent was removed and the crude material purified by flash chromatography (petroleum ether/EtOAc, 2:1) to give **9** in 98% yield (48 g) as a white solid. TLC (petroleum ether/EtOAc 3:2): $R_f = 0.45$; α:β ≈ 1:1. $[\alpha]_D$ +15.8 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 3.40–4.17, 4.18–4.30, 4.35–4.65 (m, 6 H, 6a, 6b, 5a, 5b-H), 5.05 (d, 0.5 H, $J_{1,2}$ = 7.8 Hz, 1b-H), 5.40–5.78, 5.88–6.01 (m, 6 H, 2a, 2b, 3a, 3b, 4a, 4b-H), 6.15 (d,

0.5 H, $J_{1,2}$ = 8.2 Hz, 1a), 7.20–7.65, 7.76–8.15 (m, 35 H, Ph). MALDI-MS (positive Mode, Matrix DHB, dioxane): $[M+Na]^+$, m/z = 1198.2; found: m/z = 1198.6, $[M+K]^+$, m/z = 1214.3; exp.: m/z = 1215.7. $C_{68}H_{54}O_{19}$ (1175.2): Calcd: C, 69.50; H, 4.63. Found: C, 69.15; H, 4.74.

5.5. 6-*O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzoyl-α/β-D-glucopyranose (10)

A solution of **9** (48 g, 40.85 mmol) in dry DMF (425 mL) was heated to 50 °C and hydrazinium acetate was added (5.4 g). After 3.5 h, the solution was diluted with CH_2Cl_2 and washed with water. The organic phase was dried over $MgSO_4$ and the solvent evaporated. The crude material was purified by flash chromatography (toluene/EtOAc 7:1) to give **10** in 78% of yield (34.12 g) as a colourless solid. TLC (toluene/EtOAc 6:1): R_f = 0.30. $[\alpha]_D$ +30.6 (c 0.34, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.14 (br s, 1 H, OH), 3.62–4.24, 4.38–4.59, 4.63–4.82 (m, 6 H, 6a, 6b, 5a, 5b-H), 4.98 (d, 1 H, $J_{1,2}$ = 7.6 Hz, 1b-H), 5.09–6.22 (m, 7 H, 1a, 2a, 2b, 3a, 3b, 4a, 4b-H), 7.20–7.65, 7.74–8.17 (m, 30 H, Ph). $C_{61}H_{50}O_{18}\cdot 0.5\ H_2O$ (1080.1): Calcd: C, 67.84; H, 4.76. Found: C, 67.80; H, 4.75.

5.6. *O*-[6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzoyl-α/β-D-glucopyranosyl] trichloroacetimidate (11)

Compound **10** (32.11 g, 29.98 mmol) was dissolved in dry CH_2Cl_2 (450 mL), and Cl_3CCN (30.5 mL, 0.3 mol) was added; subsequently DBU was added (0.45 mL). After 2 h, the solvent was removed in vacuo and the rest was purified by flash chromatography (petroleum ether/EtOAc 2:1, +1% Et_3N) to give **11** in 97% of yield (35.35 g) as a slightly yellow foam. TLC (petroleum ether/EtOAc 2:1): R_f = 0.33. $[\alpha]_D$ = +26 (c = 1, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.40–4.17, 4.18–4.30, 4.35–4.65 (m, 6 H, 6a, 6b, 5a, 5b-H), 5.05 (d, 0.5 H, $J_{1,2}$ = 7.8 Hz, 1b-H), 5.40–5.78, 5.88–6.01 (m, 6 H, 2a, 2b, 3a, 3b, 4a, 4b-H), 6.15 (d, 0.5 H, $J_{1,2}$ = 8.2 Hz, 1a), 7.20–7.65, 7.76–8.15 (m, 35 H, Ph). $C_{63}H_{50}O_{18}NCl_3\cdot 0.5\ H_2O$ (1224.5): Calcd: C. 61.80; H: 4.20; N: 1.14; found: C: 61.68, H: 4.17, N: 0.97.

5.7. 1,2-*O*-Isopropylidene-3-*O*-[6-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-2, 3, 4-tri-*O*-benzoyl-β-D-glucopyranosyl]-*sn*-glycerol (13)

To a solution of **12**[16] (4.2 mL, 1.2 equiv) and donor **11** (35 g, 28.8 mmol) in dry CH_2Cl_2 (450 mL) molecular sieves (3 Å) were added and the reaction mixture was stirred for 15 min at rt under argon atmosphere. Subsequently the reaction mixture was cooled to −30 °C and $BF_3\cdot Et_2O$ solution (0.2 equiv, 0.75 mL) was added. After 20 min, the reaction mixture was neutralized with NEt_3, evaporated to half volume, rediluted with toluene, and the solvent was removed in vacuo. The crude material was purified by flash chromatography (petroleum ether/EtOAc 2:1, +1% NEt_3) to give **13** in 80% of yield (27.3 g; 23.04 mmol). TLC (toluene/EtOAc 6:1): R_f = 0.3. $[\alpha]_D$ +3.6 (c 1, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.22, 1.26 (2s, 6 H, $C(CH_3)_2$), 3.42 (dd, 1 H, J_{gem} = 10.6 Hz, J_{vic} = 5.6 Hz, 1′-H), 3.62 (dd, 1 H, J_{gem} = 8.0 Hz, J_{vic} = 6.7 Hz, 3′-H), 3.69 (dd, 1 H, J_{gem} = 10.6 Hz, J_{vic} = 4.0 Hz, 1′-H), 3.81 (dd, 1 H, J_{gem} = 8.1 Hz, J_{vic} = 6.5 Hz, 3′-H), 3.85 (dd, 1 H, J_{gem} = 11.4 Hz, J_{vic} = 7.6 Hz, 6a-H), 3.96–4.03 (m, 1 H, 5a-H), 4.05 (dd, 1 H, J_{gem} = 11.4 Hz, J_{vic} = 1.5 Hz, 6a-H), 4.09–4.16 (m, 1 H, 5b-H), 4.42 (dd, 1 H, J_{gem} = 12.1 Hz, J_{vic} = 5.1 Hz, 6b-H), 4.59 (dd, 1 H, J_{gem} = 12.1 Hz, J_{vic} = 2.9 Hz, 6b-H), 4.71 (d, 1 H, $J_{1,2}$ = 7.9 Hz, 1a-H), 4.97 (d, 1 H, $J_{1,2}$ = 7.8 Hz, 1b-H), 5.31 (dd, 1 H, $J_{4,5}$ = $J_{4,3}$ = 9.7 Hz, 4a-H), 5.37 (dd, 1 H, $J_{2,1}$ = 8.1 Hz, $J_{2,3}$ = 9.6 Hz, 2a-H), 5.50 (dd, 1 H, $J_{2,1}$ = 8.0 Hz, $J_{2,3}$ = 9.6 Hz, 2b-H), 5.62 (dd, 1 H, $J_{4,5}$ = $J_{4,3}$ = 9.7 Hz, 4b-H), 5.78 (dd, 1 H, $J_{3,4}$ = $J_{3,2}$ = 9.6 Hz, 3a-H), 5.89 (dd, 1 H, $J_{3,4}$ = $J_{3,2}$ = 9.6 Hz, 3b-H), 7.17–7.58, 7.71–8.05 (m, 35 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 25.38, 26.41 (2 C, $C(CH_3)_2$), 62.94 (1 C, C-6b), 66.07 (1 C, C-3′), 68.51 (1 C, C-6a), 68.83 (1 C, C-1′), 69.51 (1 C, C-4b), 69.83 (1 C, C-4a), 71.67 (1 C, C-2a), 71.86 (1 C, C-2b), 72.28 (1 C, C-5b), 72.78 (2 C, C-3a, C-3b), 73.88 (1 C, C-5a), 74.09 (1 C, C-2′), 100.93 (1 C, C-1a), 101.27 (1 C, C-1b), 128.24–133.47 (42 C, Ph), 165.04–166.06 (7 C, *C*O-Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, m/z = 1208.2; found: m/z = 1208.9, $[M+K]^+$, m/z = 1224.3; found: m/z = 1225.3. $C_{67}H_{60}O_{20}$ (1185.2): Calcd: C, 67.90; H, 5.10. Found: C, 67.69; H, 5.01.

5.8. 1,2-*O*-Isopropylidene-3-*O*-[6-*O*-(-β-D-glucopyranosyl)-β-D-glucopyranosyl]-*sn*-glycerol (14)

To a solution of **13** (19.14 g, 16.15 mmol) in dry MeOH (300 mL) and dry CH_2Cl_2 (20 mL) was added NaH (0.81 g, 33.75 mmol) and stirred at rt. After 12 h, the reaction mixture was neutralized with amberlite IR-120 H^+, filtered and evaporated in vacuo with a little amount of Et_3N. The solid was dissolved in a mixture of dioxane/water and lyophilized, **14** was obtained in quantitative yield (7.37 g) as a yellow solid. TLC (EtOAc/MeOH 5:2): R_f = 0.28. $[\alpha]_D$ −19.1 (c = 1, $CHCl_3$). ^{1}H NMR (600 MHz, CD_3OD): δ = 1.31, 1.38 (2s, 6 H, $C(CH_3)_2$), 3.14–3.23 (m, 2 H, 2a, 2b-H), 3.26 (m, 1 H, 5b-H), 3.33 (m, 2 H, 3a, 3b-H), 3.42–3.48 (m, 1 H, 5a-H), 3.63 (dd, 1 H, $J_{1',2'}$ = 6.0 Hz, J_{gem} = 10.5 Hz, 1′-H), 3.65 (dd, 1 H, J_{gem} = 11.8 Hz, J_{vic} = 5.2 Hz, 6b-H), 3.76 (dd, 1 H, J_{gem} = 11.6 Hz, J_{vic} = 5.9 Hz, 6a-H), 3.81 (dd, 1 H, J_{gem} = 8.4 Hz, J_{vic} = 6.1 Hz, 3′-H), 3.86 (dd, 1 H, J_{gem} = 11.3 Hz, J_{vic} = 1.6 Hz, 6b-H), 3.89 (dd, 1 H, J_{gem} = 10.5 Hz, J_{vic} = 5.5 Hz, 1′-H), 4.07 (dd, 1 H, J_{gem} = 8.3 Hz, J_{vic} = 6.5 Hz, 3′-H), 4.14 (dd, 1 H, J_{gem} = 11.5 Hz, J_{vic} = 1.7 Hz, 6a-H), 4.29 (d, 1 H, $J_{1,2}$ = 7.8 Hz, 1a-H), 4.31 (m, 1 H, 2′-H), 4.36 (d, 1 H, $J_{1,2}$ = 7.8 Hz, 1b-H). ^{13}C NMR (150.9 MHz, CD_3OD): δ = 25.62 (1 C, $C(CH_3)_2$), 27.09 (1 C, $C(CH_3)_2$), 62.75 (1 C, C-6b), 67.75 (1 C, C-3′), 69.94 (1 C, C-6a), 71.45-71.59 (3 C, C-1′, C-4a/b, C-5b), 74.98, 75.10 (2 C, C-2a, C-2b), 75.86 (1 C, C-2′), 77.06 (1 C, C-5a), 77.85, 78.02 (3 C, C-4a/b, C-3a, C-3b), 104.68 (2 C, C-1a, C-1b), 104.92 (1 C, $C(CH_3)_2$). MALDI-MS (positive Mode, Matrix DHB, dioxane): $[M+Na]^+$, m/z = 479.4; found: m/z = 479.0, $[M+K]^+$, m/z = 495.5. Found: m/z = 494.8.

5.9. 1,2-*O*-Isopropylidene-3-*O*-[6-*O*-(6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-β-D-glucopyranosyl]-*sn*-glycerol (15)

A solution of **14** (7.35 g, 16.1 mmol) in pyridine (150 mL) was cooled to −15 °C and 5 mL TBDPS-Cl (1.2 equiv, 9.2 mmol) was added dropwise. The reaction mixture was stirred for 72 h, quenched with MeOH, evaporated in vacuo and coevaporated with toluene. After flash chromatography (EtOAc/MeOH 4:1), product **15** (10.2 g, 91 %) was obtained. TLC (EtOAc/MeOH 3:2): $R_f = 0.60$. $[\alpha]_D$ −25.5 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, CD_3OD): $\delta = 1.02$ (s, 9 H, *t*-Bu), 1.31, 1.39 (2s, 6 H, $C(CH_3)_2$), 3.18–4.42 (m, 19 H), 7.3–7.46, 7.63–7.80 (m, 10 H, Ph). MALDI-MS (positive Mode, Matrix DHB, dioxane): $[M+Na]^+$, $m/z = 717.85$; found: $m/z = 717.1$, $[M+K]^+$, $m/z = 733.95$; found: $m/z = 733.1$.

5.10. 1,2-*O*-Isopropylidene-3-*O*-[6-*O*-(2, 3, 4-tri-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl]-*sn*-glycerol (16)

To a solution of compound **15** (6.2 g, 8.92 mmol) in 180 mL of dry DMF was added benzyl bromide (10 equiv, 10.6 mL) at 10 °C. Then NaH (15 equiv, 2.1 g) was added portionwise, and the reaction mixture was stirred for 2 h at rt. The solvent was evaporated in vacuo; the residue was redissolved in EtOAc and washed two times with saturated NH_4Cl solution. The organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. After flash chromatography (petroleum ether/EtOAc 6:1), compound **16** (7.28 g, 66%) was obtained. TLC (petroleum ether/EtOAc 4:1): $R_f = 0.5$. $[\alpha]_D$ +9.3 (*c* 1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.04$ (s, 9 H, *t*-Bu), 1.30, 1.35 (2s, 6 H, $C(CH_3)_2$), 3.29 (m, 1 H, 5b-H), 3.42 (m, 1 H, 1′/3′-H), 3.43 (m, 1 H, 2a-H), 3.46 (m, 1 H, 4a-H), 3.50 (m, 1 H, 2b-H), 3.57 (m, 1 H, 5a-H), 3.63 (m, 1 H, 3b-H), 3.64 (m, 1 H, 1′/3′-H), 3.65 (m, 1 H, 3a-H), 3.68 (m, 1 H, 6a-H), 3,75 (dd, 1 H, $J_{4,5} = J_{4,3} = 9.4$ Hz, 4b-H), 3.69–3.95 (m, 4 H, 1′/3′-H, 6b-H, 1′/3′-H), 4.16 (m, 1 H, 2′-H), 4.22 (m, 1 H, 6a-H), 4.37 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1a-H), 4.46 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1b-H), 4.51–4.55, 4.65–4.83, 4.85–4.95, 4.97–5.03 (m, 12 H, CH_2Ph), 7.10–7.43, 7.65–7.79 (m, 40 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 19.28$ (1 C, $C(CH_3)_3$), 25.35 (1 C, $C(CH_3)_2$), 26.79 (4 C, $C(CH_3)_2$, $C(CH_3)_3$), 62.69 (1 C, C-6b), 66.51 (1 C, C-1′/3′), 68.25 (1 C, C-6a), 70.16 (1 C, C-1′/3′), 74.29 (1 C, C-2′), 74.83–76.3 (8 C, CH_2Ph, C-5a, C-5b), 77.59 (1 C, C-4b), 78.09 (1 C, C-4a), 82.13 (1 C, C-2a), 82.41 (1 C, C-2b), 84.65 (1 C, C-3a), 84.87 (1 C, C-3b), 103.69 (1 C, C-1a), 104.03 (1 C, C-1b), 127.58–138.54 (40 C, Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1258.6$; found: $m/z = 1257.7$, $[M+K]^+$, $m/z = 1274.7$; found: $m/z = 1273.9$. $C_{76}H_{86}O_{13}Si$ (1235.6): Calcd: C, 73.88; H, 7.02. Found: C, 73.95; H, 7.34.

5.11. 3-*O*-[6-*O*-(2,3,4-Tri-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl]-*sn*-glycerol (17)

To a solution of **16** (4 g, 3.24 mmol) in THF (100 mL), 200 mL of 75% CH_3COOH in water was added and stirred for 1.5 h at 80 °C. The solvent was removed in vacuo and the rest was coevaporated twice with toluene. Purification by flash chromatography (petroleum ether/EtOAc 2:1) gave compound **17** (3.02 g) in a 78% yield as a colourless foam. TLC (petroleum ether/EtOAc 2:1): $R_f = 0.25$. $[\alpha]_D$ +9.4 (*c* 1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.05$ (s, 9 H, *t*-Bu), 2.0 (br s, 1 H, OH), 2.8 (br s, 1 H, OH), 3.31–3.35 (m, 1 H, 5b-H), 3.38–3.45 (m, 3 H, 1′/3′-H, 4a-H, 2a-H), 3.50 (dd, 1 H, $J_{2,3} = J_{2,1} = 8.5$ Hz, 2b-H), 3.56 (m, 1 H, 1′-H, 3′-H), 3.62, 3.65 (m, 2 H, 1′/3′-H), 3.64 (m, 1 H, 5a-H), 3.65 (m, 1 H, 3b-H), 3.68 (m, 1 H, 3a-H), 3.70 (m, 1 H, 6a-H), 3.73 (m, 1 H, 2′-H), 3.74 (m, 1 H, 4b-H), 3.92 (m, 2 H, 6b-H), 4.18–4.23 (m, 1 H, 6a-H), 4.37 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1a-H), 4.48 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1b-H), 4.51–4.56, 4.65–4.99 (m, 12 H, CH_2Ph), 7.12–7.46, 7.66–7.77 (m, 40 H, Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1218.5$; found: $m/z = 1217.2$, $[M+K]^+$, $m/z = 1234.6$; found: $m/z = 1234.1$. $C_{73}H_{82}O_{13}Si$ (1195.5): Calcd: C, 73.34; H, 6.91. Found: C, 73.06; H, 6.91.

5.12. 1,2-Di-*O*-myristoyl-3-*O*-[6-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-*tert*-butyldiphenylsilyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl]-*sn*-glycerol (18)

To a solution of compound **17** (3.5 g, 2.93 mmol) in dry THF (140 mL), Et_3N (4.5 mL, 11 equiv) and myristoyl chloride (4.76 mL, 6 equiv) were added. The reaction mixture was stirred at 50 °C; after 4 h it was diluted with EtOAc and washed with saturated NH_4Cl solution. The organic phase was dried over $MgSO_4$, and the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/EtOAc 8:1) yielded compound **18** (3.84 g, 81%) as a colourless syrup. TLC (petroleum ether/EtOAc 5:1): $R_f = 0.6$. $[\alpha]_D$ +5 (*c* = 1, CH_2Cl_2). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.86$–0.87 (t, 6 H, Me), 1.04 (s, 9 H, *t*-Bu), 1.09–1.38 (s, 40 H, CH_2-chain), 1.45–1.62 (m, 4 H, $COCH_2C\underline{H_2}R$), 2.12–2.28 (m, 4 H, $COCH_2CH_2R$), 3.30 (m, 1 H, 5b-H), 3.41 (dd, 1 H, $J_{2,3} = J_{2,1} = 8.5$ Hz, 2a-H), 3.47 (m, 2 H, 1′/3′-H, 4a-H), 3.50 (m, 1 H, 2b-H), 3.55 (m, 1 H, 5a-H), 3.62 (m, 1 H, 3b-H), 3.65 (m, 1 H, 6a-H), 3.76 (dd, 1 H, $J_{4,5} = J_{4,3} = 9.4$ Hz, 4a-H), 3.92 (m, 1 H, 6b-H), 3.96 (m, 1 H, 1′/3′-H), 4.09–4.15 (m, 1 H, 1′/3′-H), 4.18–4.26 (m, 2 H, 1′/3′-H, 6a-H), 4.33 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1a-H), 4.42 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1b-H), 4.50-4.55, 4.64-4.84, 4.86-4.95, 4.97-5.03 (m, 12 H, CH_2Ph), 5.13 (m, 1 H, 2′-H), 7.10-7.43, 7.64-7.77 (m, 40 H, Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1639.2$; found: $m/z = 1638.5$, $[M+K]^+$, $m/z = 1655.3$; found:$m/z = 1654.5$. $C_{101}H_{134}O_{15}Si$ (1616.2): Calc.: C, 75.06; H, 8.36. Found: C, 74.81; H, 8.67.

5.13. 1,2-Di-*O*-myristoyl-3-*O*-[6-*O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl]-*sn*-glycerol (19)

Compound **18** (3.84 g, 2.38 mmol) was dissolved in dry THF (200 mL), CH_3COOH (0.6 mL, 4 equiv) and TBAF (1 M solution, 9.5 mL, 4 equiv) were added and the reaction mixture was stirred for 72 h at 40 °C. The

reaction mixture was diluted with AcOEt (500 mL) and washed with half saturated NH_4Cl solution (300 mL), the organic phase was dried over $MgSO_4$ and the solvent was evaporated in vacuo. Purification by flash chromatography (petroleum ether/EtOAc 4:1 → 3:1) yielded compound **19** (2.48 g, 76%) as a white solid. TLC (petroleum ether/EtOAc 3:1): $R_f = 0.45$. Mp 89.4 °C. $-[\alpha]_D$ +11.9 (*c* 1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.84–0.93$ (t, 6 H, Me), 1.11–1.38 (s, 40 H, CH_2-chain), 1.47–1.63 (m, 4 H, $COCH_2CH_2R$), 2.02–2.14 (s, 1 H, OH), 2.14–2.29 (m, 4 H, $COCH_2CH_2R$), 3.31–3.36 (m, 1 H, 5b-H), 3.37–3.48 (m, 3 H, 2a-H, 2b-H, 4a-H), 3.48–3.57 (m, 3 H, 5a-H, 4b-H, 1′-H), 3.60-3.73 (m, 4 H, 3a-H, 3b-H, 6a-H, 6b-H), 3.81–3.87 (m, 1 H, 6b-H), 3.92 (dd, 1 H, $J_{gem} = 10.9$ Hz, $J_{vic} = 4.5$ Hz, 1′-H), 4.08 (dd, 1 H, $J_{gem} = 11.1$ Hz, $J_{vic} < 1$ Hz, 6a-H), 4.11–4.17 (m, 1 H, 3′-H), 4.25 (dd, 1 H, $J_{gem} = 11.9$ Hz, $J_{vic} = 3.3$ Hz, 3′-H), 4.31 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1a-H), 4.45 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1b-H), 4.51–4.56, 4.61–4.70, 4.73–4.87, 4.89–4.96 (m, 12 H, CH_2Ph), 5.13–5.19 (m, 1 H, 2′-H), 7.13–7.37 (m, 30 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 14.10–34.23$ (26 C, CH_2-chain), 62.04 (1 C, C-6b), 62.69 (1 C, C-3′), 68.05 (1 C, C-1′), 68.81 (1 C, C-6a), 69.87 (1 C, C-2′), 74.69-77.0 (8 C, C-5a, C-5b, CH_2Ph), 77.58 (1 C, C-4b), 77.79 (1 C, C-4a), 81.88 (1 C, C-2a), 82.08 (1 C, C-2b), 84.50, 84.55 (2 C, C-3a, C-3b), 103.75 (1 C, C-1a), 103.93 (1 C, C-1b), 127.61–138.43 (36C, Ph), 172.97, 173.29 (2 C, $COCH_2CH_2R$). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 1400.8; found: *m*/*z* = 1399.9, $[M+K]^+$, *m*/*z* = 1416.9; found: *m*/*z* = 1417.2. $C_{85}H_{116}O_{15}$ (1377.8): Calcd: C, 74.10; H, 8.49. Found: C, 74.06; H, 8.61.

5.14. [Benzyloxy]-[diisopropylamino]-[1,2-di-*O*-myristoyl-3-*O*-{6-*O*-(2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-glucopyranosyl}-*sn*-glycero]phosphane (3)

Compound **19** (0.7 g, 0.508 mmol) was dried for 1 h together with tetrazole (21.4 mg, 0.6 eq) in high vacuum. Under argon atmosphere benzyloxybis-(diisopropylamino)-phosphane (215 mg, 1.3 equiv) dissolved in 10 mL of dry CH_2Cl_2 was added. After 30 min of stirring at rt the tetrazole was dissolved, the reaction mixture was stirred for 1.5 h; then the mixture was diluted with CH_2Cl_2 and poured over a saturated $NaHCO_3$solution. The organic phase was dried over $MgSO_4$ and removed in vacuo below 30 °C. Fast purification over flash silica gel (petroleum ether/EtOAc 5:1, +1% NEt_3) yielded compound **3** (650 mg, 79%) after one co-evaporation with toluene as a colourless syrup. TLC (petroleum ether/EtOAc 5:1, +1% NEt_3): $R_f = 0.9$. $[\alpha]_D$ +7.5 (*c* 1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.80–0.92$ (t, 6 H, Me), 1.06–1.38 (s, 40 H, CH_2-chain), 1.45–1.62 (m, 4 H, $COCH_2CH_2R$), 2.10–2.58 (m, 4 H, $COCH_2CH_2R$), 3.38 (m, 1 H, 2a-H), 3.39 (m, 1 H, 5b-H), 3.41 (m, 1 H, 4a-H), 3.42 (m, 1 H, 2b-H), 3.43 (m, 1 H, 3′-H), 3.51 (m, 1 H, 5a-H), 3.54/3.59 (m, 1 H, 4b-H), 3.61 (m, 2 H, 3a-H, 3b-H), 3.65 (m, 3 H, $NCH(CH_3)_2$, 6a-H), 3.79, 3.89, 4.00 (m, 2 H, 6b-H), 3.92 (m, 1 H, 3′-H), 4.07–4.24 (m, 3 H, 6a-H, 1′-H), 4.28 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1a-H), 4.43 (d, 1 H, $J_{1,2} = 7.8$ Hz, 1b-H), 4.45–4.52, 4.62–4.84, 4.84–4.97 (m, 14 H, CH_2Ph), 5.08–5.15 (m, 1 H, 2′-H), 7.06–7.44 (m, 35 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 14.11–34.23$ (30 C, 2 ($NCH(CH_3)_2$), CH_2-chain), 43.1, 43.18 (2 C, 2 ($NCH(CH_3)_2$)), 62.46, 62.70 (2 C, C-1′, C-6b), 65.21–65.39 (1 C, $POCH_2Ph$), 67.99 (1 C, C-3′), 68.41 (1 C, C-6a), 69.81 (1 C, C-2′), 74.66–77.0 (8 C, C-5a, C-5b, CH_2Ph), 77.74/77.85 (1 C, C-4b), 78.05 (1 C, C-4a), 81.91–82.15 (2 C, C-2a, C-2b), 84.51–84.78 (2 C, C-3a, C-3b), 103.65 (1 C, C-1a), 103.92–103.98 (1 C, C-1b), 126.84–138.61 (42 C, Ph), 172.88, 173.24 (2 C, $COCH_2CH_2R$). ^{31}P NMR (242.9 MHz, $CDCl_3$): $\delta = 149.79$, 149.89 (2s, 1 P). $C_{98}H_{136}NO_{16}P$ (1615.1): Calcd: C, 72.88; H, 8.49; N, 0.87. Found: C, 72.61; H, 8.69; N, 0.88.

5.15. Hexaphosphate 21

Compound **20**[13] (494 mg, 0.202 mmol) and phosphite amide **3** (424 mg, 1.3 equiv) were coevaporated each with dry CH_2Cl_2 (10 mL) and dried for 1 h in high vacuum. Compound **3** was dissolved in 15 mL of dry CH_2Cl_2 and was added, under argon atmosphere, to compound **20**; tetrazole (29 mg, 2 equiv, dried previously for 1 h in high vacuum) was also added. The reaction mixture was stirred at rt under argon atmosphere. After 70 min, *t*-BuO_2H (0.6 mL) was added dropwise and the reaction mixture was stirred for another 35 min. CH_2Cl_2 was added and the mixture was washed with saturated $NaHCO_3$ solution, the organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. Purification by flash chromatography (toluene/acetone 1:1) gave compound **21** (602 mg, 75%) as a colourless syrup, which was stored at −20 °C. TLC (toluene/acetone 1:1): $R_f = 0.35$, $R_f = 0.42$. $[\alpha]_D$ +12.1 (*c* 1, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.81–0.92$ (t, 6 H, Me), 1.11–1.35 (m, 40 H, CH_2-chain), 1.44–1.61 (m, 4 H, $COCH_2CH_2R$), 1.84–1.98 (m, 3 H, NHAc), 2.09–2.25 (m, 4 H, $COCH_2CH_2R$), 3.32 (m, 1 H, 5a/b-H), 3.33 (m, 1 H, 2a-H), 3,35 (m, 1 H, 1′-H), 3.37 (m, 1 H, 5a/b-H), 3.38 (m, 1 H, 2b-H), 3.43, 3.44 (m, 2 H, 4a-H, 4b-H), 3.49 (m, 2 H, 18-H), 3.57 (m, 2 H, 3a-H, 3b-H), 3.58-3.60 (m, 3 H, 6c-H, 6a-H), 3.63 (m, 4 H, 5-H, 8-H, 11-H, 14-H), 3.64 (m, 1 H, 4c-H), 3.67 (m, 12 H, OMe), 3.68 (m, 1 H, 3c-H), 3.69 (m, 1 H, 2-H), 3.72 (m, 1 H, 17-H), 3.75 (m, 1 H, 5c-H), 3.83 (m, 1 H, 1′-H), 3.96, 4.04 (m, 18 H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H), 4.06, 4.15 (m, 2 H, 3′-H), 4.08 (m, 1 H, 6a-H), 4.07, 4.17 (m, 4 H, 16-H, 6b-H), 4.20 (m, 1 H, 1a-H), 4.35 (m, 1 H, 2c-H), 4.41 (m, 1 H, 1b-H), 4.74 (m, 1 H, 1c-H), 4.29–4.91 (m, 30 H, CH_2Ph), 4.93 (m, 12 H, $POCH_2Ph$), 5.06 (m, 1 H, 2′-H), 6.69–6.80 (m, 8 H, Ph_{MPM}), 7.03–7.34 (m, 93 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 14.11$ (2 C, Me), 23.1 (1 C, NHAc), 24.84 (2 C, $COCH_2CH_2R$), 22.68/29.14–31.91 (20 C, CH_2-chain), 34.04, 34.22 (2 C, $COCH_2R$), 52.8 (1 C, C-2c), 55.17 (4 C, OMe), 63.2 (1 C, C-3′), 65.5–67.2 (12 C, $CH_{2\text{-Glyc}}$, C-6b), 68.1 (1 C, C-1′), 68.5 (1 C, C-6c), 68.6 (1 C, C-6a), 69.0 (1 C, C-18), 69.5 (6 C, $POCH_2Ph$), 69.8 (1 C, C-2′), 72 (1 C, C-5c), 73.5 (1 C, C-5a/b), 75 (1 C, C-4a/b), 75.4 (4 C, C-5, C-8, C-11, C-14), 72-77.0 (15 C, CH_2Ph), 76.8 (1 C, C-17), 77.5 (1 C, C-4c), 78.2 (1 C, C-2), 81.2 (1 C, C-3c), 81.8

(2 C, C-2a, C-2b), 84.6 (2 C, C-3a, C-3b), 100.05 (1 C, C-1c), 103.1 (1 C, C-1a), 103.6 (1 C, C-1b). MALDI-MS (positive Mode, Matrix *p*-nitroaniline+NaI, THF): $[M+Na]^+$, $m/z = 4002.3$; found: $m/z = 4000.0$. $C_{220}H_{271}NO_{54}P_6$ (3979.3): Calcd: C, 66.40; H, 6.86; N, 0.35. Found: C, 66.40; H, 7.09; N, 0.36.

5.16. Hexaphosphate 22

Compound **21** (417 mg, 0.105 mmol) was dissolved in acetonitrile/toluene/water (60:3:4, 20 mL) and cooled to −10 °C. $Ce(NH_4)_2(NO_3)_6$ (1.15 g, 20 equiv) was added portionwise and the reaction mixture was stirred for 20 min at −10 °C, the cooling bath was removed and the reaction mixture was stirred for another 30–40 min (TLC-monitoring). After this time, the reaction mixture was diluted with EtOAc and washed with saturated $NaHCO_3$ solution, the organic phase was dried over $MgSO_4$ and evaporated in vacuo. Fast purification on silica gel (toluene/acetone 1:1 → 1:3) yielded compound **22** (246 mg, 67%) as a colourless syrup, which was stored at −20 °C. TLC (toluene/acetone 1:1): $R_f = 0.40$, $R_f = 0.45$. $[\alpha]_D$ +13.6 (*c* 0.5, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.82$–0.94 (t, 6 H, Me), 1.09–1.38 (m, 40 H, CH_2-chain), 1.44–1.59 (m, 4 H, $COCH_2CH_2R$), 1.85–1.99 (m, 3 H, NHAc), 2.09–2.26 (m, 4 H, $COCH_2CH_2R$), 3.33 (m, 2 H, 2a-H, 5a/b-H), 3.37 (m, 1 H, 2b-H), 3,38 (m, 1 H, 1′-H), 3.39 (m, 1 H, 5a/b-H), 3.45 (m, 2 H, 4a-H, 4b-H), 3.52 (m, 2 H, 18-H), 3.57 (m, 2 H, 3a-H, 3b-H), 3.59 (m, 3 H, 6c-H, 6a-H), 3.67 (m, 1 H, 4c-H), 3.68 (m, 1 H, 3c-H), 3.73 (m, 2 H, 2-H, 17-H), 3.78 (m, 1 H, 5c-H), 3.85 (m, 1 H, 1′-H), 3.96, 4.07 (m, $CH_{2\text{-Glyc}}$), 4.05 (m, 4 H, 1-H, 3-H), 4.08, 4.16 (m, 2 H, 3′-H), 4.00–4.26 (m, 4 H, 6b-H, 16-H), 4.21 (m, 1 H, 1a-H), 4.31 (m, 1 H, 2c-H), 4.34–4.92 (m, 22 H, CH_2Ph), 4.42 (m, 1 H, 1b-H), 4.82 (m, 1 H, 1c-H), 5.01 (m, 12 H, $POCH_2Ph$), 5.07 (m, 1 H, 2′-H), 7.04–7.46 (m, 85 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 14.1$ (2 C, Me), 23.0 (1 C, NHAc), 24.9 (2 C, $COCH_2\underline{C}H_2R$), 22.7/29.1–29.7/31.9 (20 C, CH_2-chain), 34.0, 34.2 (2 C, $COCH_2R$), 52,8 (1 C, C-2c), 62.9 (1 C, C-3′), 65.8 (2 C, C-1, C-3), 66.0-68.2 (1′-C, $CH_{2\text{-Glyc}}$-OH, C-6b, C-16), 68.5 (1 C, C-6c), 69.0 (1 C, C-18), 69.8 (6 C, $POCH_2Ph$), 69.9 (1 C, C-2′), 71.8 (1 C, C-5c), 72.2–76.0 (11 C, CH_2Ph), 73.8 (1 C, C-5a/b), 75.2 (1 C, C-4a/b), 76.6 (2 C, C-2, C-17), 77.1 (1 C, C-4a/b), 78.0 (1 C, C-4c), 78.1 (1 C, C-5a/b), 81.0 (1 C, C-3c), 81.9 (2 C, C-2a, C-2b), 84.5 (2 C, C-3a, C-3b), 99.8 (1 C, C-1c), 103.9 (1 C, C-1a), 104.1 (1 C, C-1b). FAB-MS (positive Mode): $[M+Na]^+$, $m/z = 3519.7$; gef.: $m/z = 3522$. MALDI-MS (positive Mode, Matrix *p*-nitroaniline+NaI, THF): $[M+Na]^+$, $m/z = 3519.7$; found: $m/z = 3518$. $C_{188}H_{237}NO_{50}P_6H_2O$ (3514.7): Calcd: C, 64.25; H, 6.85; N, 0.40. Found: C, 64.33; H, 7.31; N, 0.40.

5.17. Hexaphosphate 23a

Compound **22** (197 mg, 0.056 mmol), PyBOP (585 mg, 20 equiv) and Z-D-Ala triethylammonium salt (365 mg, 20 equiv) were dried separately for 3 h in high vacuum. After this time, **22** was dissolved in dry CH_2Cl_2 (15 mL), Z-D-Ala triethylammonium salt and PyBOP were added. *N*-Methyl imidazole (180 μL, 40 equiv) was added dropwise and the reaction mixture was stirred for 2.5–3 h at rt under argon atmosphere. The reaction mixture was diluted with CH_2Cl_2 and washed with saturated NH_4Cl solution. The organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. Purification by flash chromatography (toluene/acetone 3:1) and second column (toluene/acetone 3:1) yielded **23a** as a mixture of diastereomers (170 mg, 70%) as a colourless syrup which was stored at −20 °C. TLC (toluene/acetone 1:1): $R_f = 0.71$, $R_f = 0.75$. $[\alpha]_D$ +15 (*c* 0.15, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6 H, Me), 1.12–1.41 (m, 52 H, CH_2-chain, Ala-Me), 1.46–1.59 (m, 4 H, $COCH_2CH_2R$), 1.86–1.99 (m, 3 H, NHAc), 2.10–2.26 (m, 4 H, $COCH_2CH_2R$), 3.33 (m, 1 H, 5a/b-H), 3.34 (m, 1 H, 2a-H), 3.35 (m, 1 H, 5a/b-H), 3.36 (m, 1 H, 1′-H), 3.39 (m, 1 H, 2b-H), 3.44, 3.46 (m, 2 H, 4a-H, 4b-H), 3.52 (m, 2 H, 18-H), 3.57 (m, 2 H, 3a-H, 3b-H), 3.59 (m, 2 H, 6c-H), 3.67 (m, 1 H, 6a-H), 3.69 (m, 2 H, 4c-H, 3c-H), 3.74 (m, 2 H, 2-H, 17-H), 3.76 (m, 1 H, 5c-H), 3.85 (m, 1 H, 1′-H), 4.01 (m, 20 H, 1-H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H), 4.07 (m, 1 H, 16-H), 4.08 (m, 1 H, 3′-H), 4.10 (m, 1 H, 6a-H), 4.16 (m, 1 H, 3′-H), 4.18 (m, 3 H, 6b-H, 16-H), 4.21 (m, 1 H, 1a-H), 4.33 (m, 4 H, $CHNHCbz$), 4.34 (m, 1 H, 2c-H), 4.32-4.92 (m, 22 H, CH_2Ph), 4.43 (m, 1 H, 1b-H), 4.79 (m, 1 H, 1c-H), 4.98 (m, 4 H, CH_2Cbz), 4.99 (m, 12 H, $POCH_2Ph$), 5.05 (m, 4 H, CH_2Cbz), 5.08 (m, 1 H, 2′-H), 5.09 (m, 4 H, 5, 8, 11, 14-H), 5.56–6.14 (NH), 7.04–7.46 (m, 105 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): $\delta = 14.4$ (2 C, Me), 18.3 (4 C, Ala-Me), 23.1 (1 C, NHAc), 25.2 (2 C, $COCH_2CH_2R$), 29.8 (2 0C, CH_2-chain), 34.3, 34.5 (2 C, $COCH_2R$), 49.9 (4 C, $CHNHCbz$), 53.0 (1 C, C-2c), 63.0 (1 C, C-3′), 65.1 (10 C, C-1, C-3, C-4, C-6, C-7, C-9, C-10, C-12, C-13, C-15), 66.9 (1 C, C-6b), 67.0 (4 C, CH_2Cbz), 67.5 (1 C, C-16), 68.3 (1 C, C-1′), 68.9 (2 C, C-6a, C-6c), 69.1 (1 C, C-18), 70.0 (1 C, C-2′), 70.1 (6 C, $POCH_2Ph$), 71.0 (4 C, CH-Ala), 72.0 (1 C, C-5c), 72.2-76.0 (11 C, CH_2Ph), 73.8 (1 C, C-5a/b), 75.3 (1 C, C-4a/b), 76.8 (2 C, C-2, C-17), 77.1 (1 C, C-4a/b), 77.9 (1 C, C-4c), 78.0 (1 C, C-5a/b), 81.1 (1 C, C-3c), 82.1 (2 C, C-2a, C-2b), 84.8 (2 C, C-3a, C-3b), 100.2 (1 C, C-1c), 103.8 (1 C, C-1a), 104.1 (1 C, C-1b). MALDI-MS (positive Mode, Matrix *p*-nitroaniline+NaI, MeOH): $[M+Na]^+$, $m/z = 4340$; found: $m/z = 4336$. $C_{232}H_{281}N_5O_{62}P_6$ (4317.6): Calcd: C, 64.54; H, 6.56; N, 1.62. Found: C, 64.34; H, 6.75; N, 1.51.

5.18. Target molecule 1a

The diastereomers **23a** (83 mg, 0.019 mmol) were dissolved in CH_2Cl_2/MeOH/H_2O (7.5:7.5:1.5, 6 mL), treated with Pearlman's catalyst (10% in weight) and under hydrogen atmosphere, with a H_2-filled balloon, was stirred overnight at rt. The reaction mixture was filtered through Celite, washed with CH_2Cl_2/MeOH/H_2O (7.5:7.5:1.5, 2 mL) and the filtrate was diluted with 0.1 M NH_4OAc-buffer (pH 4.8). The solvent was lyophilized and purified using hydrophobic interaction HPLC on octylsepharose. After lyophilization, compound **1a** (20 mg, 47%) was obtained as white powder. $[\alpha]_D$ +6.9

(c 0.13, H_2O). ^1H NMR (600 MHz, D_2O): δ = 0.82–0.94 (m, 6 H, Me), 1.16–1.43 (m, 40 H, CH_2-chain), 1.55–1.69 (m, 16 H, Ala-Me, $COCH_2CH_2R$), 2.11 (s, 3 H, NHAc), 2.29–2.47 (m, 4 H, $COCH_2CH_2R$), 3.27–4.59 (m, 54 H), 5.10 (br s, 1 H, 1c-H), 5.31–5.43 (m, 5 H, CH-Ala, 2′-H). ^{13}C NMR (150.9 MHz, D_2O): δ = 14.2 (2 C, Me), 15.9 (4 C, Ala-Me), 22.6 (1 C, NHAc), 25.4 (2 C, $COCH_2CH_2R$), 30.5 (20 C, CH_2-chain), 34.5 (2 C, $COCH_2CH_2R$), 49.2 (4 C, $CHNH_3^+$), 54.0 (1 C, C-2c), 60.9 (1 C, C-6c), 62.4 (1 C, C-1β), 64.0 (C–$CH_{2\text{-Glyc}}$, C-3′), 65.5 (C–$CH_{2\text{-GlycGlcNAc}}$), 66.8 (1 C, C-16), 68.2 (1 C, C-1′), 70.3 (1 C, C-4c), 71.1 (1 C, C-17), 71.4 (1 C, C-3c), 72.4 (1 C, C-5c), 73.4 (2 C, C-2a, C-2b), 74.4 (4 C, CH-Ala), 75.9 (2 C, C-3a, C-3b), 76.2 (1 C, C–CH_{GlcNAc}), 97.2 (1 C, C-1c). MALDI-MS (negative Mode, Matrix THAP, CH_3CN/H_2O 3:2): $[M-H]^-$, m/z = 2247.9; found: m/z = 2248.5; $[(M\text{-}Ala)-H]^-$, m/z = 2176.9; found: m/z = 2177.6.

5.19. Compound 23b

Compound **23b** was synthesized following the same method as for **23a**, but using (Z)-L-alanine triethylammonium salt. Compound **22** (250 mg, 0.072 mmol) yielded **23b** (190 mg, 62%) as a colourless syrup. TLC (toluene/acetone 1:1): R_f = 0.71, R_f = 0.75. $[\alpha]_D$ +8.5 (c 0.18, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$): δ = 0.87 (t, 6 H, Me), 1.10–1.40 (m, 52 H, CH_2-chain, Ala-Me), 1.46–1.62 (m, 4 H, $COCH_2CH_2R$), 1.82–1.99 (m, 3 H, NHAc), 2.09–2.26 (m, 4 H, $COCH_2CH_2R$), 3.32 (m, 1 H, 5a/b-H), 3.33 (m, 1 H, 2a-H), 3,35 (m, 1 H, 1′-H), 3.37 (m, 1 H, 5a/b-H), 3.38 (m, 1 H, 2b-H), 3.43, 3.45 (m, 2 H, 4a-H, 4b-H), 3.50 (m, 2 H, 18-H), 3.57 (m, 2 H, 3a-H, 3b-H), 3.59 (m, 2 H, 6c-H), 3.63 (m, 1 H, 6a-H), 3.67 (m, 1 H, 4c-H), 3.68 (m, 1 H, 3c-H), 3.71 (m, 2 H, 2-H, 17-H), 3.76 (m, 1 H, 5c-H), 3.83 (m, 1 H, 1′-H), 4.01 (m, 20 H, 1-H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H), 4.07 (m, 2 H, 3′-H, 16-H), 4.09 (m, 1 H, 6a-H), 4.14 (m, 1 H, 6b-H), 4.15 (m, 1 H, 3′-H), 4.17 (m, 1 H, 16-H), 4.21 (m, 2 H, 6b-H, 1a-H), 4.33 (m, 4 H, CHNHCbz), 4.33–4.92 (m, 22 H, CH_2Ph), 4.34 (m, 1 H, 2c-H), 4.43 (m, 1 H, 1b-H), 4.79 (m, 1 H, 1c-H), 4.97 (m, 4 H, CH_2Cbz), 4.99 (m, 12 H, $POCH_2$Ph), 5.05 (m, 4 H, CH_2Cbz), 5.07 (m, 1 H, 2′-H), 5.09 (m, 4 H, 5, 8, 11, 14-H), 5.59–6.17 (NH), 7.04–7.45 (m, 105 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 14.4 (2 C, Me), 18.3 (4 C, Ala-Me), 23.1 (1 C, NHAc), 25.2 (2 C, $COCH_2CH_2R$), 22.9, 29.8, 32.1 (20 C, CH_2-chain), 34.2, 34.5 (2 C, $COCH_2R$), 50.1 (4 C, CHNHCbz), 53.0 (1 C, C-2c), 63.4 (1 C, C-3′), 65.5 (10 C, C-1, C-3, C-4, C-6, C-7, C-9, C-10, C-12, C-13, C-15), 67.2 (4 C, CH_2Cbz), 67.3 (1 C, C-6b), 67.9 (1 C, C-16), 68.5 (1 C, C-1′), 69.2 (2 C, C-6a, C-6c), 69.3 (1 C, C-18), 70.0 (1 C, C-2′), 70.3 (6 C, $POCH_2$Ph), 71.3 (4 C, CH-Ala), 72.2 (1 C, C-5c), 72.6–76.5 (11 C, CH_2Ph), 74.0 (1 C, C-5a/b), 75.5 (1 C, C-4a/b), 77.0 (2 C, C-2, C-17), 77.8 (1 C, C-4a/b), 78.2 (1 C, C-4c), 78.5 (1 C, C-5a/b), 81.6 (1 C, C-3c), 82.7 (2 C, C-2a, C-2b), 85.2 (2 C, C-3a, C-3b), 100.2 (1 C, c-1c), 104.0 (1 C, C-1a), 104.4 (1 C, C-1b). MALDI-MS (positive Mode, Matrix p-nitroaniline+NaI, THF): $[M+Na]^+$, m/z = 4340; found: m/z = 4337.6. $C_{232}H_{281}N_5O_{62}P_6$ (4317.6): Calcd: C, 64.48; H, 6.51; N, 1.62. Found: C, 64.11; H, 6.68; N, 1.54.

5.20. Target molecule 1b

Compound **1b** was obtained using the same procedure as for **1a**. Compound **23b** (83 mg, 0.019 mmol) yielded **1b** (17 mg, 40%) as white powder. $[\alpha]_D$ +5.1 (c 0.17, H_2O). ^1H NMR (600 MHz, D_2O): δ = 0.80–0.96 (br s, 6 H, Me), 1.13–1.46 (m, 40 H, CH_2-chain), 1.53–1.77 (m, 16 H, Ala-Me, $COCH_2CH_2R$), 2.10 (s, 3 H, NHAc), 2.27–2.49 (m, 4 H, $COCH_2CH_2R$), 3.25–4.62 (m, 54 H), 5.09 (br s, 1 H, 1c-H), 5.29–5.46 (m, 5 H, CH-Ala, 2′-H). ^{13}C NMR (150.9 MHz, D_2O): δ = 16.7 (2 C, Me), 18.2 (4 C, Ala-Me), 25.0 (1 C, NHAc), 27.6 (2 C, $COCH_2CH_2R$), 25.5/33.1/34.9 (20 C, CH_2-chain), 37.0 (2 C, $COCH_2CH_2R$), 51.7 (4 C, $CHNH_3^+$), 56.5 (1 C, C-2c), 63.7 (1 C, C-6c), 65.1 (1 C, C-18), 66.3 (1 C, C-3′), 66.6 (C–$CH_{2\text{-Glyc}}$), 68.1 (C–$CH_{2\text{-GlycGlcNAc}}$), 69.4 (1 C, C-16), 73.1 (1 C, C-4c), 73.7 (1 C, C-17), 74.0 (1 C, C-3c), 75.2 (1 C, C-5c), 76.2 (2 C, C-2a, C-2b), 77.0 (4 C, CH-Ala), 78.7 (2 C, C-3a, C-3b), 79.0 (1 C, CH_{GlcNAc}), 99.9 (1 C, C-1c). MALDI-MS (negative Mode, Matrix THAP, CH_3CN/H_2O 3:2): $[M-H]^-$, m/z = 2247.9; found: m/z = 2245.5; $[(M\text{-}Ala)-H]^-$, m/z = 2176.9; found: m/z = 2174.8. MALDI-MS (positive Mode, Matrix HCCA, H_2O): $[M+H]^+$, m/z = 2249.9; found: m/z = 2249.9.

5.21. 1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-(4-methoxybenzyl)-3-*O*-monomethoxytrityl-*sn*-glycerol (25)

Compound **24**[13] (3.56 g, 7.9 mmol) was dissolved in CH_2Cl_2/pyridine (1:1, 80 mL), monomethoxytrityl chloride was added and the reaction mixture was stirred overnight at rt. The solvent was removed in vacuo and the residue was dissolved in EtOAc, washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, and the solvent was removed in vacuo. Flash chromatography (petroleum ether/EtOAc 20:1) yielded **25** (5.6 g, 98%) as a colourless crystals. TLC (petroleum ether/EtOAc 7:1): R_f = 0.39. Mp 118 °C. $-[\alpha]_D$ +2.3 (c 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 0.96 (s, 9 H, t-Bu), 3.18–3.33 (m, 2 H, CH_{2Glyc}) 3.67–3.91 (m, 9 H, CH_{2Glyc}, CH_{Glyc}, OMe), 4.59 (s, 2 H, $C\underline{H}_2$Ph), 6.74–6.91 (m, 4 H, $Ph_{MPM,\ MMTr}$), 7.17–7.71 (m, 24 H, Ph). $C_{47}H_{50}O_5Si$ (723.0): Calcd: C, 78.08; H, 6.97. Found: C, 78.12; H, 7.02.

5.22. 2-*O*-(4-Methoxybenzyl)-3-*O*-monomethoxytrityl-*sn*-glycerol (6)

Compound **25** (5.5 g, 7.6 mmol) was dissolved in THF (100 mL) and TBAF (9.1 mL, 1 M, 1.2 equiv) was added dropwise. The reaction mixture was stirred for 2 h at rt, and the solution was diluted with EtOAc, washed with water, saturated $NaHCO_3$ solution and dried over $MgSO_4$. The solvent was removed in vacuo. Flash chromatography (petroleum ether/EtOAc 3:1 → 2:1) yielded **6** (3.5 g, 96%) as a colourless syrup. TLC (petroleum ether/EtOAc 6:1): R_f = 0.07. $[\alpha]_D$ +25 (c 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.95–2.05 (m, 1 H, OH), 3.17–3.36 (m, 2 H, CH_{2Glyc}) 3.59–3.91 (m, 6 H, CH_{2Glyc}, CH_{Glyc}, OMe), 4.47 (d, 1 H, J_{gem} = 11.3 Hz, CH_2Ph), 4.62 (d, 1 H, J_{gem} = 11.3 Hz, CH_2Ph), 6.79–6.97 (m, 4 H, $Ph_{MPM,\ MMTr}$), 7.18–7.56 (m, 14 H, Ph). $C_{31}H_{32}O_5$

(484.6): Calcd: C, 76.66; H, 6.61. Found: C, 76.34; H, 6.86.

5.23. 3-*O*-Allyl-2-*O*-benzyl-1-*O*-*tert*-butyldiphenylsilyl-*sn*-glycerol (27)

To a solution of **26**[13] (15 g, 40.5 mmol) in DMF were added benzyl bromide (7.24 mL, 60.7 mmol) and NaH (2 g, 2 equiv) portionwise. The reaction mixture was stirred for 3 h. The solvent was evaporated in vacuo and the residue was dissolved in EtOAc, washed with saturated NH_4Cl solution and dried over $MgSO_4$. The solvent was removed in vacuo. Flash chromatography (petroleum ether/EtOAc 30:1) yielded **27** (13.4 g, 72%) as a colourless syrup. TLC (petroleum ether/EtOAc 7:1): $R_f = 0.49$. $[\alpha]_D$ −1.3 (*c* 1, acetone). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.05$ (s, 9 H, *t*-Bu), 3.52–3.87, 3.96–4.10 (m, 7 H, CH_{2Glyc}–H, CH_{Glyc}–H, CH_2CHCH_2), 4.64 (s, 2 H, CH_2Ph), 5.11–5.35 (m, 2 H, CH_2CHCH_2), 5.81–6.01 (m, 1 H, CH_2CHCH_2), 7.21–7.53, 7.61–7.81 (m, 15 H, Ph). $C_{29}H_{36}O_3Si$ (460.7): Calcd: C, 75.61; H, 7.88. Found: C, 75.37; H, 7.90.

5.24. 2-*O*-Benzyl-1-*O*-*tert*-butyldiphenylsilyl-*sn*-glycerol (28)

Compound **27** (13.3 g, 28.9 mmol) was dissolved in dry EtOH (300 mL), DBU (648 μL, 0.15 equiv) and $(Ph_3P)_3RhCl$ (8 g, 0.3 equiv) were added and the reaction mixture was stirred at 90 °C. After 15 min, the propenyl intermediate was formed ($R_f = 0.76$, toluene/acetone 1.5:1); the solvent was removed in vacuo and the residue was dissolved in a solution of 1 M HCl/acetone (1:9, 200 mL) and stirred at 70 °C for 15 min. The solution was neutralized with NEt_3, diluted with EtOAc and washed with saturated $NaHCO_3$ solution. After drying with $MgSO_4$, the solvent was removed in vacuo. Flash chromatography (petroleum ether/EtOAc 3:1) yielded **28** (9.2 g, 76 %) as a yellow syrup. The analytical data are in accordance with those reported in the literature.[21]

5.25. 2-*O*-Benzyl-3-*O*-monomethoxytrityl-1-*O*-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (29)

Compound **29** was obtained as described for **25** from compound **28** (9.15 g, 21.75 mmol) and monomethoxytrityl chloride (8.2 g, 26.55 mmol). Flash chromatography (PE/EE 20:1) yielded **29** (14.77 g, 98%) as a colourless syrup. TLC (petroleum ether/EtOAc 7:1): $R_f = 0.48$. $[\alpha]_D$ +1.9 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 0.96$ (s, 9 H, *t*-Bu), 3.21–3.35 (m, 2 H, CH_{2Glyc}–H) 3.69–3.85 (m, 6 H, $CH_{2\text{-}Glyc}$–H, CH_{Glyc}–H, OMe), 4.67 (s, 2 H, CH_2Ph_{MPM}), 6.74–6.83 (m, 2 H, Ph_{MPM}), 7.15–7.51, 7.58–7.69 (m, 27 H, Ph). $C_{46}H_{48}O_4Si$ (693.0): Calcd: C, 79.73; H, 6.98. Found: C, 79.80; H, 7.09.

5.26. MPM2-*O*-Benzyl-3-*O*-monomethoxytrityl-*sn*-glycerol (30)

Compound **29** (16 g, 23.1 mmol) was dissolved in THF (300 mL) and TBAF-solution (27 mL, 1 M in THF, 1.2 equiv) was added dropwise. The reaction mixture was stirred for 3 h, diluted with EtOAc and washed with saturated $NaHCO_3$ solution, the organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. Flash chromatography (petroleum ether/EtOAc 3:1) yielded **30** (9.86 g, 98%) as a colourless syrup. TLC (petroleum ether/EtOAc 7:1): $R_f = 0.13$. $[\alpha]_D$ +23.6 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.98$–2.05 (m, 1 H, OH), 3.23 (dd, 1 H, $J_{gem} = 9.9$ Hz, $J_{vic} = 4.9$ Hz, $CH_{2\text{-}Glyc}$–H) 3.30 (dd, 1 H, $J_{gem} = 9.9$ Hz, $J_{vic} = 4.7$ Hz, CH_{2Glyc}–H), 3.62–3.79 (m, 3 H, $CH_{2\text{-}Glyc}$, CH_{Glyc}), 3.79 (s, 3 H, OMe), 4.54 (d, 1 H, $J_{gem} = 11.7$ Hz, CH_2Ph), 4.69 (d, 1 H, $J_{gem} = 11.7$ Hz, CH_2Ph), 6.80–6.88 (m, 2 H, Ph_{MPM}), 7.19–7.52 (m, 17 H, Ph). $C_{30}H_{30}O_4 \cdot 0.25H_2O$ (459.1): Calcd: C, 78.49; H, 6.70. Found: C, 78.45; H, 6.82.

5.27. [Benzyloxy]-[diisopropylamino]-[2-*O*-benzyl-3-*O*-monomethoxytrityl-*sn*-glycero]phosphane (7)

Compound **7** was obtained as described for **3**. Tetrazole (108 mg, 0.6 equiv), **30** (2.03 g, 7.46 mmol), benzyloxy-bis-(diisopropyl amine)-phosphane (1.37 g, 1.2 equiv). Flash chromatography (petroleum ether/EtOAc 8:1, +1% NEt_3) yielded **7** (2.4 g, quant.) as a colourless oil. TLC (petroleum ether/EtOAc 7:1, 1% NEt_3): $R_f = 0.54$. $[\alpha]_D$ −0.5 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.10$–1.30 (m, 12 H, $CH(CH_3)_2$), 3.19–3.34 (m, 2 H, $CH_{2\text{-}Glyc}$), 3.47–3.91 (m, 8 H, OMe, $CH(CH_3)_2$, $CH_{2\text{-}Glyc}$), 4.51–4.80 (m, 4 H, $POCH_2Ph$, CH_2Ph), 6.73–6.89 (m, 2 H, Ph_{MMTr}), 7.13–7.58 (m, 22 H, Ph). $C_{43}H_{50}NO_5P$ (691.8): Calcd: C, 74.65; H, 7.28; N, 2.02. Found: C, 74.64; H, 7.35; N, 2.02.

5.28. Compound 31

Compound **31** was synthesized following the general coupling procedure. Compound **6** (3.59 g, 7.41 mmol), tetrazole (780 mg, 11.14 mmol) and phosphite amide **5**[13] (5.6 g, 1.1 equiv) were dissolved in dry CH_2Cl_2 (150 mL). (TLC, petroleum ether/EtOAc: 7:1, +1% NEt_3; $R_f = 0.22$). Oxidation with *t*-BuO_2H (4 mL) and flash chromatography (petroleum ether/EtOAc 3:1) yielded **31** (7.89 g, 98%) as a colourless syrup. TLC (petroleum ether/EtOAc 1:1): $R_f = 0.69$. $[\alpha]_D$ −0.4 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.01$ (s, 9 H, *t*-Bu), 3.13-3.22 (m, 2 H, 6-H), 3.58–3.88 (m, 13 H, 1, 2-H, 5-H, OMe), 4.03–4.31 (m, 4 H, 3-H, 4-H), 4.41–4.57 (m, 4 H, CH_2-Ph), 4.92–5.01 (m, 2 H, $POCH_2$-Ph), 6.72–6.88 (m, 6 H, $Ph_{MPM,\ MMTr}$), 7.10–7.50, 7.60–7.70 (m, 31 H, Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, $m/z = 1110.3$; found: $m/z = 1110.4$. $C_{65}H_{71}O_{11}PSi \cdot 0.5\ H_2O$ (1096.3): Calcd: C, 71.21; H, 6.62. Found: C, 71.10; H, 6.68.

5.29. 3-*O*-[2-*O*-(4-Methoxybenzyl)-3-*O*-monomethoxytrityl-*sn*-glycero-(1)-benzylphospho]-2-*O*-(4-methoxybenzyl)-*sn*-glycerol (32)

Comoound **32** was synthesized following the general deprotection procedure. **31** (7.78 g, 7.16 mmol), TBAF-solution (8.60 mL, 1.2 equiv), THF (150 mL) and flash chromatography (petroleum ether/EtOAc 1:1 → 1:3)

yielded **32** (5 g, 82%) as a colourless syrup. TLC (toluene/acetone 1:1): $R_f = 0.61$. $[\alpha]_D$ +12.5 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 3.16–3.25$ (m, 2 H, 6-H), 3.48–3.89 (m, 13 H, 1-H, 2-H, 5-H, OMe), 4.01–4.30 (m, 4 H, 3-H, 4-H), 4.39–4.60 (m, 4 H, CH_2-Ph), 4.96–5.05 (m, 2 H, POCH_2-Ph), 6.76–6.91 (m, 6 H, $Ph_{MPM,\ MMTr}$), 7.15–7.49 (m, 21 H, Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 871.9; found: *m*/*z* = 872.2. $C_{49}H_{53}O_{11}P{\cdot}0.5H_2O$ (857.9): Calcd: C, 68.60; H, 6.35. Found: C, 68.61; H, 6.40.

5.30. Diphosphate 33

Compound **33** was synthesized following the general coupling procedure. **32** (5.13 g, 6.04 mmol), tetrazole (635 mg, 9.07 mmol) and phosphite amide **5**[13] (5.0 g, 1.2 equiv) were dissolved in dry CH_2Cl_2 (120 mL). (TLC petroleum ether/EtOAc: 1:1, +1% NEt_3, $R_f = 0.68$). Oxidation with *t*-BuO_2H (3.5 mL). Flash chromatography (petroleum ether/EtOAc 2:1 → 1:1) yielded **33** (8.4 g, 96%) as a colourless syrup. TLC (petroleum ether/EtOAc 1:1): $R_f = 0.35$. $[\alpha]_D$ −1.1 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.02$ (s, 9 H, *t*-Bu), 3.14–3.22 (m, 2 H, 9-H), 3.59–3.82 (m, 17 H, 1-H, 2-H, 5-H, 8-H, OMe), 3.90–4.32 (m, 8 H, 3-H, 4-H, 6-H, 7-H), 4.39–4.54 (m, 6 H, CH_2-Ph), 4.90–5.02 (m, 4 H, POCH_2-Ph), 6.69–6.88 (m, 8 H, $Ph_{MPM,\ MMTr}$), 7.09–7.48, 7.59–7.70 (m, 38 H, Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]_+$, *m*/*z* = 1474.6; found: *m*/*z* = 1475.9. $C_{83}H_{92}O_{17}P_2Si$ (1451.6): Calcd: C, 68.67; H, 6.39. Found: C, 68.34; H, 6.37.

5.31. Diphosphate 34

Compound **34** was synthesized following the general deprotection procedure. **33** (8.3 g, 5.72 mmol) TBAF-solution (6.9 mL, 1.2 equiv), THF (150 mL) and flash chromatography (toluene/acetone 2.5:1) yielded **34** (6.34 g, 91%) as a colourless syrup. TLC (toluene/acetone 1:1): $R_f = 0.32$. $[\alpha]_D$ +5.1 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 2.62–2.71$ (br s, 1 H, OH), 3.14–3.23 (m, 2 H, 9-H), 3.50–3.87 (m, 17 H, 1-H, 2-H, 5-H, 8-H, OMe), 3.90–4.28 (m, 8 H, 3-H, 4-H, 6-H, 7-H), 4.40–4.59 (m, 6 H, CH_2-Ph), 4.91–5.07 (m, 4 H, POCH_2-Ph), 6.73–6.89 (m, 8 H, $Ph_{MPM,\ MMTr}$), 7.11–7.47 (m, 28 H, Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 1236.2; found: *m*/*z* = 1236.0, $[M+K]^+$, *m*/*z* = 1252.3; found: *m*/*z* = 1252.3. $C_{67}H_{74}O_{17}P_2$ (1213.2): Calcd: C, 66.33; H, 6.15. Found: C, 66.54; H, 6.37.

5.32. Triphosphate 35

Compound **35** was synthesized following the general coupling procedure. Compound **34** (6.30 g, 5.19 mmol), tetrazole (563 mg, 8.04 mmol) and phosphite amide **5**[13] (4.42 g, 1.2 equiv) were dissolved in dry CH_2Cl_2 (120 mL). (TLC toluene/acetone 3:1, +1% NEt_3, $R_f = 0.62$). Oxidation with *t*-BuO_2H (3.3 mL). Flash chromatography (toluene/acetone 4.5:1) yielded **35** (8.82 g, 94%) as a colourless syrup. TLC (toluene/acetone 3:1): $R_f = 0.48$. $[\alpha]_D$ −0.7 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.02$ (s, 9 H, *t*-Bu), 3.13–3.22 (m, 2 H, 12-H), 3.56–3.88 (m, 21 H, 1-H, 2-H, 5-H, 8-H, 11-H, OMe), 3.90–4.32 (m, 12 H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H), 4.39–4.58 (m, 8 H, CH_2-Ph), 4.89–5.08 (m, 6 H, POCH_2-Ph), 6.69–6.89 (m, 10 H, $Ph_{MPM,\ MMTr}$), 7.10–7.50, 7.59–7.71 (m, 45 H, Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 1838.97; found: *m*/*z* = 1838.8, $[M+K]^+$, *m*/*z* = 1855.1; found: *m*/*z* = 1856.3. $C_{101}H_{113}O_{23}P_3Si$ (1816.0): Calcd: C, 66.80; H, 6.27. Found: C, 67.30; H, 6.15.

5.33. Triphosphate 36

To a solution of triphosphate **35** (8.66 g, 4.77 mmol) in CH_2Cl_2 (50 mL) and MeOH (100 mL), CSA was added (222 mg, 0.95 mmol, 0.2 equiv); the reaction mixture was stirred for 2 h at rt. After neutralization with NEt_3, the solvent was removed in vacuo and coevaporated with toluene. Flash chromatography (toluene/acetone 2.5:1) yielded **36** (6.8 g, 92%) as a colourless syrup. TLC (toluene/acetone 2.5:1): $R_f = 0.30$. $[\alpha]_D$ −7.5 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.02$ (s, 9 H, *t*-Bu), 2.73–3.0 (br s, 1 H, OH), 3.50–3.86 (m, 20 H, 1-H, 2-H, 5-H, 8-H, 11-H, 12-H, OMe), 3.91–4.34 (m, 12 H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H), 4.39–4.60 (m, 8 H, CH_2-Ph), 4.93–5.10 (m, 6 H, POCH_2-Ph), 6.71–6.90 (m, 8 H, $Ph_{MPM,\ MMTr}$), 7.10–7.47, 7.59–7.70 (m, 33 H, Ph). MALDI-MS (positive Mode, Matrix DHB, THF): $[M+Na]^+$, *m*/*z* = 1566.6; found: *m*/*z* = 1567.3, $[M+K]^+$, *m*/*z* = 1582.7; found: *m*/*z* = 1582.4. $C_{81}H_{97}O_{22}P_3Si$ (1543.6): Calcd: C, 63.02; H, 6.33. Found: C, 63.02; H, 6.58.

5.34. Tetraphosphate 37

Compound **37** was synthesized following the general coupling procedure. Compound **36** (2.5 g, 1.62 mmol), tetrazole (170 mg, 2.43 mmol) and phosphite amide **7** (1.35 g, 1.2 equiv) were dissolved in dry CH_2Cl_2 (60 mL). (TLC toluene/acetone 3:1, +1% NEt_3, $R_f = 0.62$). Oxidation with *t*-BuO_2H (2 mL). Flash chromatography (toluene/acetone 2:1) yielded **37** (3.34 g, 96%) as a colourless syrup. TLC (toluene/acetone 2:1): $R_f = 0.42$. $[\alpha]_D$ −1.1 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): $\delta = 1.02$ (s, 9 H, *t*-Bu), 3.17–3.25 (m, 2 H, 15-H), 3.59–3.81 (m, 19 H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, OMe), 3.89–4.32 (m, 16 H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H), 4.38–4.51 (m, 8 H, CH_2-Ph), 4.51–4.61 (m, 2 H, CH_2-Ph), 4.90–5.04 (m, 8 H, POCH_2-Ph), 6.69–6.88 (m, 10 H, $Ph_{MPM,\ MMTr}$), 7.09–7.49, 7.58–7.69 (m, 55 H, Ph). $C_{118}H_{132}O_{28}P_4Si$ (2150.3): Calcd: C, 65.91; H, 6.19. Found: C, 66.09; H, 6.16.

5.35. Tetraphosphate 38

Compound **38** was synthesized following the general deprotection procedure. **37** (3.3 g, 1.54 mmol), TBAF-solution (1.85 mL, 1.2 equiv), THF (60 mL). Flash chromatography (toluene/acetone 2:1 → 1:1) yielded **38** (2.78 g, 95%) as a colourless syrup. TLC (toluene/acetone 1:1):

R_f = 0.53. $[\alpha]_D$ +2.5 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 2.75–2.99 (br s, 1 H, OH), 3.14–3.26 (m, 2 H, 15-H), 3.50–3.84 (m, 19 H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, OMe), 3.89–4.29 (m, 16 H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H), 4.49–4.63 (m, 10 H, CH_2-Ph), 4.89–5.10 (m, 8 H, POCH_2-Ph), 6.70–6.92 (m, 10 H, $Ph_{MPM,\ MMTr}$), 7.10–7.49 (m, 45 H, Ph). $C_{102}H_{114}O_{28}P_4$·toluene (2004.0): Calcd: C, 65.33; H, 6.14. Found: C, 65.41; H, 6.12.

5.36. Pentaphosphate 39

Compound **39** was synthesized following the general coupling procedure. Compound **38** (2.71 g, 1.42 mmol), tetrazole (150 mg, 2.14 mmol) and phosphite amide **4**[13] (1.78 g, 1.2 equiv) were dissolved in dry CH_2Cl_2 (60 mL). (TLC toluene/acetone 3:1, +1% NEt_3, R_f = 0.62). Oxidation with *t*-BuO_2H (2 mL). Flash chromatography (toluene/acetone 2:1 → 1:3) yielded **39** (3.60 g, 96%) as a colourless syrup. TLC (toluene/acetone 1:1): R_f = 0.52, R_f = 0.60. $[\alpha]_D$ +12.0 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.02 (s, 9 H, *t*-Bu), 1.90–2.03 (m, 3 H, NHAc), 3.10–3.26 (m, 3 H, 18, 6c-H), 3.41–3.83 (m, 27 H, 3c-H, 4c-H, 5c-H, 6c-H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, 17-H, OMe), 3.88–4.80 (m, 38 H, 1c-H, 2c-H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H, 16-H, CH_2-Ph), 4.85–5.05 (m, 10 H, POCH_2-Ph), 6.68–6.88 (m, 10 H, $Ph_{MPM,\ MMTr}$), 7.02–7.71 (m, 75 H, Ph). $C_{157}H_{176}NO_{38}P_5Si$ (2868.0): Calcd: C, 65.75; H, 6.19; N, 0.49. Found: C. 66.11; H, 6.44; N, 0.48.

5.37. Pentaphosphate 40

To a solution of **39** (3.53 g, 1.23 mmol) in CH_2Cl_2 (60 mL) and MeOH (20 mL), CSA was added (86 mg, 0.37 mmol, 0.3 equiv); the reaction mixture was stirred for 2 h at rt. After neutralization with NEt_3, the solvent was removed in vacuo and coevaporated with toluene. Flash chromatography (toluene/acetone 1:1 → 1:3) yielded **40** (2.93 g, 92%) as a colourless syrup. TLC (toluene/acetone 1:1): R_f = 0.55. $[\alpha]_D$ +7.6 (*c* 1, $CHCl_3$). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.02 (s, 9 H, *t*-Bu), 1.89–2.02 (m, 3 H, NHAc), 3.11–3.23 (m, 1 H, 6c-H), 3.40–3.85 (m, 26 H, 3c-H, 4c-H, 5c-H, 6c-H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, 17-H, 18-H, OMe), 3.88–4.80 (m, 38 H, 1c-H, 2c-H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H, 16-H, CH_2-Ph), 4.85–5.11 (m, 10 H, POCH_2-Ph), 6.68–6.89 (m, 8 H, Ph_{MPM}), 7.01–7.46, 7.46–7.70 (m, 63 H, Ph). $C_{137}H_{160}NO_{37}P_5Si$ (2595.7): Calcd: C, 63.39; H, 6.21; N, 0.54. Found: C, 63.10; H, 6.46; N, 0.55.

5.38. Pentaphosphate 41

Compound **40** (433 mg, 0.17 mmol) was dissolved in THF (15 mL) and TBAF solution (0.2 mL, 1.2 equiv) was added. After 45 min, the reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and water, and after drying over $MgSO_4$ the solvent was removed in vacuo. Flash chromatography (toluene/acetone 1:1.5) yielded **41** (335 mg, 85%) as a colourless syrup. TLC (toluene/acetone 1:1.5): R_f = 0.09, R_f = 0.20, R_f = 0.33. $[\alpha]_D$ +15.5 (*c* = 0.75, acetone). ^1H NMR (250 MHz, $CDCl_3$): δ = 1.86–2.01 (m, 3 H, NHAc), 2.80–3.20 (br s, 2 H, OH), 3.41–3.83 (m, 27 H, 3c-H, 4c-H, 5c-H, 6c-H, 1-H, 2-H, 5-H, 8-H, 11-H, 14-H, 17-H, 18-H, OMe), 3.89–4.84 (m, 38 H, 1c-H, 2c-H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H, 16-H, CH_2-Ph), 4.90–5.10 (m, 10 H, POCH_2-Ph), 6.71–6.89 (m, 8 H, Ph_{MPM}), 7.08–7.45 (m, 53 H, Ph).

5.39. Heptaphosphate 42

Compound **42** was synthesized following the general coupling procedure. Compound **41** (320 mg, 0.136 mmol), tetrazole (19 mg, 0.272 mmol) and phosphite amide **3** (570 mg, 2.5 equiv) were dissolved in dry CH_2Cl_2 (10 mL). Oxidation was achieved with *t*-BuO_2H (1 mL). Flash chromatography (toluene/acetone 3:1 → 1.5:1) yielded **42** (520 mg,71%) as a colourless syrup. TLC (toluene/acetone 2:1): R_f = 0.44, R_f = 0.51, R_f = 0.59. $[\alpha]_D$ +10.34 (*c* 0.75, acetone). ^1H NMR (600 MHz, $CDCl_3$): δ = 0.80–0.91 (t, 12 H, Me), 1.09–1.35 (m, 80 H, CH_2-chain), 1.44–1.52 (m, 8 H, $COCH_2CH_2R$), 1.87–1.98 (m, 3 H, NHAc), 2.09–2.28 (m, 8 H, $COCH_2CH_2R$), 3.31, 3.39 (m, 4 H, 2a-H, 2b-H), 3.33 (m, 2 H, 5a/b-H), 3,36 (m, 2 H, 1′-H), 3.38 (m, 2 H, 5a/b-H), 3.45 (m, 4 H, 4a-H, 4b-H), 3.56 (m, 1 H, 6c-H), 3.58 (m, 4 H, 3a-H, 3b-H), 3.60 (m, 2 H, 6a-H), 3.62 (m, 6 H, 2-H, 5-H, 8-H, 11-H, 14-H, 17-H), 3.64 (m, 1 H, 6c-H), 3.66 (m, 12 H, OMe), 3.68 (m, 1 H, 3c-H), 3.69 (m, 1 H, 4c-H), 3.77 (m, 1 H, 5c-H), 3.84 (m, 2 H, 1′-H), 3.95, 4.03 (m, 24 H, 1-H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H, 16-H, 18-H), 4.06 (m, 2 H, 3′-H), 4.10 (m, 2 H, 6a-H), 4.15 (m, 2 H, 3′-H), 4.18 (m, 2 H, 6b-H), 4.21 (m, 2 H, 1a-H), 4.23 (m, 2 H, 6b-H), 4.33 (m, 1 H, 2c-H), 4.42 (m, 2 H, 1b-H), 4.69/4.73 (m, 1 H, 1c-H), 4.32-4.91 (m, 40 H, CH_2Ph), 4.94 (m, 14 H, POCH_2Ph), 5.07 (m, 2 H, 2′-H), 6.68–6.81 (m, 8 H, Ph_{MPM}), 7.05–7.35 (m, 123 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 53.0 (1 C, C-2c), 55.7 (4C, OMe), 63.1 (2 C, C-3′), 66.0 (12 C, C–$CH_{2\text{-Glyc}}$), 66.8 (2 C, C-6b), 68.4 (2 C, C-1′), 68.9 (1 C, C-6c), 69.0 (2 C, C-6a), 69.7 (7 C, PO*C*H_2Ph), 69.9 (2 C, C-2′), 72.0–78.0 (20 C, *C*H_2Ph), 72.2 (1 C, C-5c), 74.0 (2 C, C-5a/b), 75.5 (2 C, C-4a/b), 75.9 (6 C, C-2, C-5, C-8, C-11, C-14, C-17), 77.3 (2 C, C-4a/b), 78.0 (1 C, C-4c), 78.3 (2 C, C-5a/b), 81.4 (1 C, C-3c), 82.2 (4 C, C-2a, C-2b), 84.9 (4 C, C-3a, C-3b), 101.0 (1 C, C-1c), 104.0 (2 C, C-1a), 104.2 (2 C, C-1b). MALDI-MS (positive Mode, Matrix *p*-nitroaniline+NaI, THF): $[M+Na]^+$, *m*/*z* = 5440.1; found: *m*/*z* = 5445.6. $C_{305}H_{384}NO_{71}P_7$ (5417.1): Calcd: C, 67.62; H, 7.14; N, 0.26. Found: C, 67.35; H, 7.32; N, 0.20.

5.40. Heptaphosphate 43

Compound **43** was synthesized following the same procedure used for **22**. Compound **42** (476 mg, 0.088 mmol) was dissolved in CH_3CN/toluene/H_2O (60:3:4, 15 mL). Flash chromatography (toluene/acetone 1:1. → 1:3) yielded **43** (330 mg, 76%) as a colourless syrup. TLC (toluene/acetone 1:1.5): R_f = 0.44, R_f = 0.53. $[\alpha]_D$ +12.6 (*c* 0.5, acetone). ^1H NMR (600 MHz, $CDCl_3$): δ = 0.80–0.92 (t, 12 H, Me), 1.08–1.36 (m, 80 H, CH_2-chain), 1.43–1.59 (m, 8 H, $COCH_2CH_2R$), 1.82–1.97

(m, 3 H, NHAc), 2.11–2.27 (m, 8 H, COCH_2CH$_2$R), 3.34 (m, 2 H, 5a/b-H), 3.35 (m, 4 H, 2a-H, 2b-H), 3.37 (m, 2 H, 5a/b-H), 3.38 (m, 2 H, 1′-H), 3.46 (m, 2 H, 4a/b-H), 3.47 (m, 2 H, 4a/b-H), 3.56 (m, 1 H, 6c-H), 3.58 (m, 4 H, 3a-H, 3b-H), 3.59 (m, 2 H, 6a-H), 3.64 (m, 1 H, 6c-H), 3.67 (m, 2 H, 2-H, 17-H), 3.68 (m, 1 H, 3c-H), 3.69 (m, 1 H, 4c-H), 3.80 (m, 1 H, 5c-H), 3.85 (m, 2 H, 1′-H), 3.94 (m, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H), 3.99 (m, 4 H, 1-H, 3-H, 16-H, 18-H), 4.07 (m, 2 H, 3′-H), 4.08 (m, 4 H, 1-H, 3-H, 16-H, 18-H), 4.10 (m, 2 H, 6a-H), 4.15 (m, 2 H, 3′-H), 4.16 (m, 2 H, 6b-H), 4.22 (m, 2 H, 1a-H), 4.24 (m, 2 H, 6b-H), 4.31 (m, 1 H, 2c-H), 4.34-4.93 (m, 32 H, CH_2Ph), 4.42 (m, 2 H, 1b-H), 4.83 (m, 1 H, 1c-H), 5.01 (m, 14 H, POCH_2Ph), 5.08 (m, 2 H, 2′-H), 7.05–7.47 (m, 115 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 53.0 (1 C, C-2c), 63.0 (2 C, C-3′), 66.0 (4 C, C-1, C-3, C-16, C-18), 66.8 (2 C, C-6b), 68.3 (2 C, C-1′), 68.4 ($CH_{2\text{-Glyc}}$-OH), 68.9 (1 C, C-6c), 69.0 (2 C, C-6a), 70.0 (7 C, POCH_2Ph), 70.1 (2 C, C-2′), 72.0-76.4 (16 C, CH_2Ph), 72.1 (1 C, C-5c), 73.9 (2 C, C-5a/b), 75.5 (2 C, C-4a/b), 76.0 (2 C, C-2, C-17), 77.3 (2 C, C-4a/b), 78.1 (1 C, C-4c), 78.3 (2 C, C-5a/b), 81.1 (1 C, C-3c), 82.2 (4 C, C-2a, C-2b), 84.9 (4 C, C-3a, C-3b), 99.9 (1 C, C-1c), 104.0 (2 C, C-1a), 104.2 (2 C, C-1b). MALDI-MS (positive Mode, Matrix *p*-nitroaniline+NaI, THF): $[M+Na]^+$, m/z = 4959.5; found: m/z = 4966.1. $C_{273}H_{352}NO_{67}P_7$ (4936.5): Calcd: C, 66.42; H, 7.19; N, 0.28. Found: C, 66.30; H, 7.38; N, 0.24.

5.41. Fully protected target molecule 44

Compound **44** was synthesized following the procedure used for **23a**. Compound **43** (296 mg, 0.060 mmol) gave **44** (170 mg, 49%, mixture of diastereomers) as a colourless syrup. TLC (toluene/acetone 1:1): R_f = 0.60, R_f = 0.67. $[\alpha]_D$ +12.45 (*c* 0.48, acetone). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.80–0.94 (t, 12 H, Me), 1.07–1.38 (m, 92 H, CH_2-chain, Ala-Me), 1.45–1.59 (m, 8 H, COCH$_2$CH_2R), 1.85–1.97 (m, 3 H, NHAc), 2.11–2.28 (m, 8 H, COCH_2CH$_2$R), 3.33 (m, 2 H, 5a/b-H), 3.34 (m, 4 H, 2a-H, 2b-H), 3.36 (m, 4 H, 5a/b-H, 1′-H), 3.46 (m, 4 H, 4a-H, 4b-H), 3.58 (m, 4 H, 3a-H, 3b-H), 3.60 (m, 4 H, 6c-H, 6a-H), 3.64 (m, 2 H, 2-H, 17-H), 3.68 (m, 1 H, 3c-H), 3.70 (m, 1 H, 4c-H), 3.76 (m, 1 H, 5c-H), 3.84 (m, 2 H, 1′-H), 4,00 (m, 24 H, 1-H, 3-H, 4-H, 6-H, 7-H, 9-H, 10-H, 12-H, 13-H, 15-H, 16-H, 18-H), 4.09 (m, 2 H, 3′-H), 4.11 (m, 2 H, 6a-H), 4.15 (m, 2 H, 3′-H), 4.18 (m, 2 H, 6b-H), 4.22 (m, 2 H, 1a-H), 4.24 (m, 2 H, 6b-H), 4.33 (m, 4 H, C*H*-NHCbz), 4.35 (m, 1 H, 2c-H), 4.34-4.93 (m, 32 H, CH_2Ph), 4.42 (m, 2 H, 1b-H), 4.78 (m, 1 H, 1c-H), 4.96 (m, 4 H, CH_2-Cbz), 4.98 (m, 14 H, POCH_2Ph), 5.03 (m, 4 H, CH_2-Cbz), 5.08 (m, 2 H, 2′-H), 5.09 (m, 4 H, 5, 8, 11, 14-H), 5.76-6.18 (bs, NH), 7.03–7.46 (m, 135 H, Ph). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 49.9 (4 C, *C*H-NHCbz), 53.0 (1 C, C-2c), 63.0 (2 C, C-3′), 65.3 (12 C, C-1, C-3, C-4, C-6, C-7, C-9, C-10, C-12, C-13, C-15, C-16, C-18), 66.8 (2 C, C-6b), 67.1 (4 C, *C*H$_2$-Cbz), 68.3 (2 C, C-1′), 68.8 (1 C, C-6c), 68.9 (2 C, C-6a), 70.0 (2 C, C-2′), 70.3 (7 C, PO*C*H$_2$Ph), 71.0 (4 C, C-5, C-8, C-11, C-14), 72.0 (1 C, C-5c), 73.9 (2 C, C-5a/b), 72.5–75.9 (16 C, *C*H$_2$Ph), 73.8 (2 C, C-5a/b), 75.3 (2 C, C-4a/b), 76.1 (2 C, C-2, C-17), 77.1 (2 C, C-4a/b), 77.9 (1 C, C-4c), 78.3 (2 C, C-5a/b), 81.2 (1 C, C-3c), 82.1 (4 C, C-2a, C-2b), 84.7 (4 C, C-3a, C-3b), 100.6 (1 C, C-1c), 103.8 (2 C, C-1a), 104.2 (2 C, C-1b). MALDI-MS (positive Mode, Matrix *p*-nitroaniline+NaI, THF): $[(M-H)+2Na]^+$, m/z = 5802.3; found: m/z = 5801.3. $C_{317}H_{396}N_5O_{79}P_7$ (5757.34): Calcd: C, 66.13; H, 6.93; N, 1.22. Found: C, 65.94; H, 7.08; N, 1.20.

5.42. Target molecule 2

Compound **2** was synthesized following the procedure used for **1a**. Compound **44** (107 mg, 0.019 mmol) yielded **2** (19 mg, 16%) as white powder. ^{1}H NMR (600 MHz, D_2O): δ = 0.78–0.96 (m, 12 H, Me), 1.13–1.46 (m, 80 H, CH_2-chain), 1.55–1.74 (m, 20 H, Ala-Me, COCH$_2$CH_2R), 2.11 (s, 3 H, NHAc), 2.27–2.49 (m, 8 H, COCH_2CH$_2$R), 3.40–4.60 (m, 72 H), 5.10 (m, 1 H, 1c-H), 5.31–5.45 (m, 6 H, CH-Ala, 2′-H). ^{13}C NMR (150.9 MHz, D_2O): δ = 14.2 (4 C, Me), 15.9 (4 C, Ala-Me), 22.6 (1 C, NHAc), 25.2 (4 C, COCH$_2$*C*H$_2$R), 30.9 (40 C, *C*H$_2$-chain), 34.7 (4 C, CO*C*H$_2$R), 49.1 (4 C, *C*HNH$_3^+$, 60.9 (1 C, C-6c), 64.0 (C–$CH_{2\text{-Glyc}}$), 66.4 (C–$CH_{2\text{-Glyc}}$), 74.1 (4C, CH-Ala). MALDI-MS (negative Mode, Matrix THAP, CH_3CN/H_2O 3:2): $[M-H]^-$, m/z = 3147.0; found: m/z = 3146.0; $[(M\text{-Ala})-H]^-$, m/z = 3074.9; found: m/z = 3075.2.

5.43. Measurement of biological activity

Heparinized whole blood from healthy donors controlled by differential blood cell count was diluted 5-fold with RPMI 1640 (BioWhittaker Europe) and stimulated overnight (37 °C, 5% CO_2) in polypropylene cups with equimolar concentrations of **1a** and **2**. Cytokines were measured in the supernatants using ELISA antibody pairs against TNFα and IL-8 (Endogen, Pierce, Perbio Science, Bonn, Germany).

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